THE
AMERICAN NATURALIST

Vol. LXXXVII March-April, 1953 No. 833

INVENTIONS IN IRON METABOLISM*

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If we examine the human body for the element iron we find that the body contains about 4.5 g of iron. Practically all of this iron is not free as inorganic iron. Rather the iron is found to be present in some organic complex form attached to various proteins. Only traces of iron are present in the body of the kind that one may consider as inorganic ferrous or ferric iron. What of the distribution of iron among these various compounds? We see from table 1 that about three-fourths of the total iron is present in the body in the form of blood and muscle hemoglobin and most of the remainder of the iron is accounted for as storage iron (Granick, 1949). There is one group of iron compounds which is present in the body in very low concentrations. These are the cytochromes. The iron of the cytochromes makes up only about 1/1000 of the total iron. Yet the iron, as represented in the cytochromes, although very low as far as total iron concentration is concerned, serves a most important function—that of catalyzing oxidation or burning with oxygen. Indeed, we shall see that all of the other iron compounds of the body are present to make possible the proper functioning of this 1/1000th part of the body iron of the cytochromes.

This afternoon I would like to present to you some facts and much speculation on how protoplasm came to use iron and how evolving protoplasm in its ever increasing complexity came to invent new iron compounds to suit its newer requirements. The analysis of evolution in terms of chemical substances is not new, but I think that in the case of iron the facts at the present time lend themselves more readily to a wider scope of speculations than for most other substances of biological import.

More specifically, we will attempt to illustrate the possibility that from a knowledge of some of the properties of inorganic iron one may deduce certain hypotheses about evolution of oxidative catalysts. In addition we will discuss the conditions which were to arise during evolution that were to make necessary certain inventions in iron metabolism.

*Presented at the A.I.B.S. symposium on Biochemical Evolution at Ithaca, N. Y. September 8, 1952.

EVOLUTION OF THE IRON OXIDATIVE ENZYMES

Let us begin by classifying the iron compounds of the body into two groups, those iron compounds which appeared early in the evolution of protoplasm and those which came much later. Let us first consider the iron compounds which appeared early in evolution. The compounds which appeared, certainly by the time a single cell was formed, are the iron porphyrin or heme enzymes which today constitute only a very small fraction of the total iron of the body. One of these compounds is the very important oxidative catalyst cytochrome oxidase. You will recall that the iron porphyrin or heme enzymes are made up on the following general pattern. As shown in figure 1, their active or prosthetic group consists of a flat, red colored molecule called porphyrin at the center of which is an iron atom. Now this iron of the heme unit when it becomes attached to a particular protein becomes an enzyme. Depending on the kind of iron linkage to the particular protein, one obtains heme enzymes that possess different catalytic properties.

TABLE 1

APPROXIMATE COMPOSITION OF THE IRON-CONTAINING COMPOUNDS IN THE HUMAN (70-KILO MAN) (AFTER GRANICK, 1949)

Compounds	g.	Fe in g.	% of total Fe
Iron porphyrin (heme) compounds:			
Blood hemoglobin	900	3.0	60–70
Muscle hemoglobin (myoglobin)	40	0.13	3–5
Heme enzymes:			
Cytochrome *c*	0.78	0.004	0.1
Cytochrome Oxidase	.....	.....	.....
Catalase	.....	.....	.....
Peroxidase	.....	.....	.....
Non-heme iron compounds:			
Iron monomolecularly dispersed:			
Ferrous iron	.....	.....	.....
Siderophilin	10.0	0.004	0.1
Iron in special micelles iron hydroxide units:			
Ferritin	2–4	0.4–0.8	15
Hemosiderin	.....	.....	.....
Total iron		4–5	100

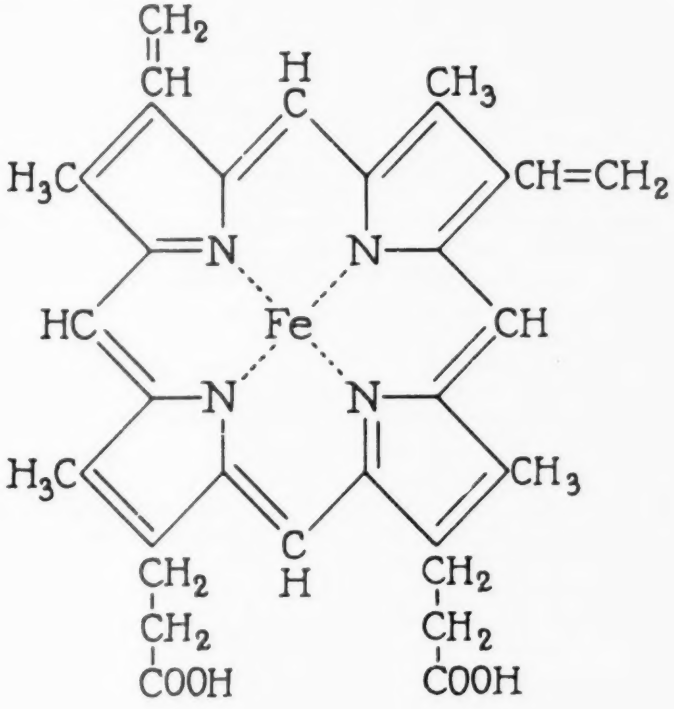
One may distinguish four general kinds of heme enzymes depending on their specific activities (Granick and Gilder, 1947). These kinds of heme enzymes are

1. Cytochrome oxidase—which activates O_2 to behave as a strong electron acceptor.
2. Catalase—which decomposes H_2O_2.
3. Peroxidase—which activates H_2O_2 to behave as a strong electron acceptor.
4. Cytochrome c—which serves to transport electrons.

Now these four catalytic properties of the heme enzymes appear at first glance to be unrelated to each other in terms of the differing activities.

However, if we compare the properties of inorganic iron compounds with the properties of the very active heme enzymes, a very interesting correlation becomes apparent. It is this: the lowly inorganic iron atom itself possesses all of these four properties of the different heme enzymes. For example, the property of electron transfer by cytochrome *c* may be represented by the reversible oxidation of ferrous to ferric iron. The activity of cytochrome oxidase may be represented by the reaction of ferrous ions with O_2. The catalase and peroxidase properties are also possessed by inorganic ferrous and ferric iron. The same inorganic iron atom then can possess all of these four catalytic activities. It is non-specialized as far as these four properties go, but its catalytic activities are rather weak compared to the catalytic activities of the heme enzymes.

For example, let us examine one of these catalytic properties of inorganic iron, say the catalase activity—the ability to split H_2O_2 molecules



Fe protoporphyrin 9

FIGURE 1

into O_2 and water; and let us see how an atom of inorganic iron may have been converted during evolution into a highly efficient and specialized catalyst. Inorganic ferrous iron has a very low catalase activity. Certain ferric hydroxides may show a hundred-fold increase in activity as compared with ferrous iron. When iron is incorporated into the protoporphyrin ring the resulting iron protoporphyrin or heme molecule may show a thousand-fold increase over the activity of inorganic iron. Now, when the heme molecule becomes attached to a specific protein, the catalase activity is about a million times that of inorganic ferrous iron itself. Thus, we see that by incorporating iron into a ring compound and then attaching this ring compound to a special protein we have obtained a selective activity, namely, that of splitting H_2O_2 and this activity has been enhanced one million-fold over that of the inorganic iron atom itself.

If we make the hypothesis that protoplasm originated from simple beginnings, conceivably from combinations that were predominantly inorganic, we have in the iron atom a link with the beginnings of protoplasm.

The most important function of the heme enzymes is that of catalyst for oxidation with O_2. Assuming that inorganic iron would be the catalyst for oxidation in early protoplasm, why was iron selected rather than a number of other possible elements? Because of the limitation of time it will not be possible to discuss the reasons why iron was most suitable. It will have to suffice here to say that two properties are essential for an efficient oxidative catalyst. First, if a catalyst is to act rapidly it should be capable of accepting one electron at a time in a thermodynamically reversible manner. Second, if the cell is to be capable of using most of the energy that will be released in oxidation with O_2 then what is required is a catalyst whose potential is as close to the potential of the O_2 electrode as possible.

Several catalysts that might have been used were the chromous-chromic, the titanous-titanic, the cuprous-cupric and the ferrous-ferric systems. The system which comes closest to satisfying the properties that are required for an oxidative catalyst under the environmental conditions of our planet is the ferrous-ferric system.

The hypothesis of conversion of inorganic iron to the heme protein catalysts is compatible with a basic evolutionary principle: namely, the conservation of function. The function of oxidation must have been a very early part of the chemistry of protoplasm, to provide the energy for synthetic reactions. It is obvious that this function of oxidation must be conserved no matter what other changes may occur in the evolution of protoplasm. Thus the simple iron compounds in early protoplasm could have functioned in essentially the same manner as the more complex iron compounds of newer protoplasm. In short, what seems to have evolved, beginning in early protoplasm with inorganic iron as catalyst, were a series of highly complicated iron enzyme catalysts that had selective and enhanced catalytic activities and that functioned with greater efficiency.

THE INVENTION OF HEMOGLOBIN

We have discussed the iron oxidative enzymes, namely, the cytochromes and other heme enzymes and have noted that they constitute only a very small but very important part of the total body iron. Let us next consider a series of iron compounds which account for the major amount of iron in the body. These iron compounds appeared relatively late in evolution, becoming of great significance at a time when protoplasm began to organize itself into larger and more complex masses of cells. Such masses of cells could no longer obtain by diffusion adequate amounts of O_2 to supply their oxidative cytochrome enzymes. The problem of supplying O_2 to body cells became especially acute at the time of the evolution of the primitive vertebrates. Although no one invention can be said to have been a major one in the development of this amazing group, it is apparent that with an adequate O_2 supply, the vertebrate body could increase in size, and the brain which requires such a high O_2 tension was enabled to enlarge. Thus the invention of hemoglobin was one of the essential requisites for vertebrate evolution and eventually made possible the transport in the blood stream of the mammal of 50 times more O_2 than could be carried by an equal volume of sea water.

Hemoglobin, like the cytochromes, is also a heme or iron porphyrin protein. It may be pictured as a protein layer cake, the globin, with four squares of red icing on the sides of the cake, representing the four heme units attached to the protein globin. Here the iron atom of a heme can combine loosely with O_2 molecules. In view of the fact that inorganic iron possesses all of the properties of the heme enzymes one might expect inorganic iron to possess the ability in a limited degree to combine with O_2 loosely. No experimental evidence is available on this point as yet. Certainly in early protoplasm there would be no particular need for storage or transport of O_2.

Now, the invention of hemoglobin was not a new one at the stage of vertebrate evolution. If we examine various invertebrates we find that hemoglobin makes a haphazard appearance throughout the phyla. We find hemoglobin appearing in certain paramecia, in various worms, molluscs, insects and even in the root nodules of leguminous plants. In some cases hemoglobin is said to serve as a temporary O_2 store in cells, but primarily it serves to transport O_2. It seems as if hemoglobin were invented time and again independently, rather than necessarily being inherited from previous phyla. This result is not too surprising if we assume that all cells of necessity had to make heme for oxidative catalysts and that every once in a while a protein of the correct configuration arose to which heme might attach to produce a particular heme protein which had the property of combining loosely with O_2.

The story of hemoglobin, the different hemoglobins, either free or enclosed within cells and the correlation between their binding of oxygen to function adequately in various organisms is one of the great chapters

in physiology and is adequately told in many textbooks (Baldwin, 1937; Florkin, 1949; Prosser, 1950). I shall not dwell upon it here. What I wish now to discuss is not hemoglobin, but rather some of the inventions that were developed to make possible the formation of adequate amounts of hemoglobin. Certain of these inventions we have become aware of only in the last 10–15 years (Granick, 1946, 1949).

INVENTIONS TO MAKE IRON AVAILABLE FOR HEMOGLOBIN SYNTHESIS

To make adequate amounts of hemoglobin, amounts that will supply enough O_2 to the traces of iron cytochrome catalysts, a biologically huge amount of iron is required, an amount of iron several hundred to a thousand times more than is contained in all the cytochrome and other heme enzymes of the body combined. We use the expression "biologically huge" in order to stress the fact that such amounts of iron are obtained only with great difficulty by organisms.

The difficulty arises primarily because of two properties of inorganic iron itself, namely, the great insolubility of ferric hydroxide and the spontaneous oxidation of ferrous iron by O_2 to the ferric state, that is, autoxidation of ferrous iron.

Let us briefly consider some of these properties of inorganic iron. The solubility of ferrous iron at body pH, that is, the solubility of ferrous iron in a neutral solution saturated with ferrous iron, is about five parts of ferrous iron per million parts of water. Although this is indeed a small amount of iron, yet it is of a significant magnitude. Ferrous ions are important because it is in this reduced state or ferrous state that iron appears to be absorbed by the cells. For example, if the intestinal tract of a vertebrate organism can be supplied with a saturated solution of ferrous iron it will slowly obtain all the iron the body requires for its growth even though this saturated solution would contain only five parts of ferrous iron per million of solution. Another property of ferrous iron that is important is its autoxidation to the ferric state. This autoxidation is negligible in acid solutions, it is rapid at the pH of the body and very rapid even in weakly alkaline solutions such as that of sea water. Now, at body pH ferric ions rapidly form ferric hydroxide molecules and these molecules of ferric hydroxide tend to combine with each other by splitting out water to form Fe-O-Fe-O-Fe bonds leading rapidly to the formation of polymerized ferric hydroxide clusters or micelles or aggregates of ferric hydroxide. This means that very few single ferric hydroxide molecules would continue to exist since the tendency is very great toward the formation of ferric hydroxide micelles. In sea water it has been estimated that the amounts of single free ferric hydroxide molecules are around 10^{-17} molal—an astronomically small figure.

Practically all the iron in the food that we consume is in the ferric state, much of it as ferric hydroxide or as loose ferric complexes. The iron in the food must be converted to the ferrous state to be absorbed. And the problem is how can ferrous iron be made from ferric iron.

To convert ferrous iron to the ferric state a reducing agent is necessary as represented in the forward reaction of the equation.

$$Fe^{+++} \underset{O_2}{\overset{\text{reducing substances}}{\rightleftharpoons}} Fe^{++}$$

However, O_2 tends to autoxidize the ferrous ion back to the ferric state as represented by the reverse reaction of the equation.

In order to maintain the iron in the ferrous state it is necessary to prevent or slow up the autoxidation with O_2. One way this can be done is to bring about the reduction in an atmosphere devoid of O_2, that is, an anaerobic atmosphere. Thus, no autoxidation of ferrous ion will occur. Another way is to bring about reduction in an aerobic atmosphere when the medium is acid. In an acid medium ferrous ion is not attacked by O_2 and is therefore relatively stable.

We may cite two examples to illustrate environmental conditions under which these properties of iron can come into play so that ferrous iron would be readily available to organisms. In stagnant pools, especially those into which water drains from acid soils, the ferrous iron content of the water would be appreciable. However, since autoxidation would be especially rapid at the alkaline pH of oxygenated sea water, ferrous iron would be present only in the immediate vicinity of a rich anaerobic mud. Thus organisms like the worm Urechis or Spirographis, which have a hemolymph stream red with hemoglobin, may be able to acquire sufficient amounts of iron for their hemoglobin by residing in a rich mud environment.

It becomes apparent that to obtain sufficient ferrous iron for hemoglobin requirements would present an especially acute problem to any forms that would exist in oxygenated sea water. The problem could be solved by creating an internal environment, say, in the intestinal tract, that would reduce the iron taken in as part of the food and keep the iron in the ferrous form until it is absorbed by the mucosal cells of the intestinal tract. Two possibilities for solving this problem are suggested by the properties of inorganic iron we have discussed. One possibility is to make a portion of the intestinal tract acid. In the presence of acid the ferric hydroxide micelles are broken down and converted to ferric ions. These ferric ions may be reduced in the acid medium by substances of the food such as the SH groups of proteins and ascorbic acid to the ferrous state. And in the acid medium the ferrous ions do not become autoxidized in the presence of O_2. Actually this is the mechanism that is used in the vertebrates and the acidity, as you know, is provided by the HCl secreted into the stomach. Another possibility would be to render a portion of the intestinal tract anaerobic perhaps by bacterial fermentative processes and let the bacteria then provide the reducing substances for the conversion of ferric iron to the ferrous state. This latter device does not appear to have been used, as far as we know. But it would be interesting to examine certain fishes which lack stomachs to see whether such a device might be operable.

As we ascend the phylogenetic tree we first encounter HCl formation in the chordates among the fishes. As far as we know, no HCl secreting

cells are known below the craniata. Now various reasons have been given for having HCl in the gastro-intestinal tract: 1) killing of bacteria, 2) prevention of bacterial putrefaction, 3) killing prey, 4) HCl requirement for proteolytic digestion—and perhaps others. But I would like to propose that one of the more important reasons for the invention of a cell that would secrete HCl in the stomach was to make available the large amount of iron that is required for production of large amounts of hemoglobin.

The need for large amounts of iron not only required that sufficient ferrous iron be made available, but required several other inventions. Arising from the fact that ferrous iron was now made available to the mucosal cells of the intestine, the necessity arose of regulating the amount of iron that was to be accumulated by the vertebrate. We become aware of the damaging effect of excessive amounts of iron in cases of hemochromatosis, where, because of hereditary lesions to the regulating mechanism, too much iron accumulates in the body. This regulating mechanism for iron differs from that say for K^+ or Na^+. As you know, the K^+ or Na^+ ions are absorbed freely, and regulation is at the port of exit, that is, in the kidney tubules. The regulating mechanism for iron is at the port of entry, that is, in the mucosal cells of the intestinal tract. No iron is excreted by the kidneys. Indeed, iron once absorbed into the body does not leave it. There is a slight loss of iron only from cells that are sloughed off from skin and intestinal tract. When hemoglobin and other heme proteins break down, a mechanism exists for splitting open the porphyrin ring and releasing the iron. The iron is conserved and is reutilized over and over again. The mechanism of regulating the entry of iron by way of the mucosal cells is not well understood. Evidence indicates that the mucosal cells permit a small steady amount of iron to be absorbed, sufficient for normal body needs; yet in cases of hemorrhage or pregnancy where demands for iron become great, the mucosal cells appear to permit more iron to be absorbed than normally.

From the mucosal cells of the intestinal tract the iron is carried around the body to be stored in the liver or to be utilized by the bone marrow. This transport of iron is not haphazard. There is another invention involved in the transport of iron; it is a special protein called "siderophilin" discovered independently by Shade and Caroline (1946), and by Holmberg and Laurell (1945). This protein serves to capture and transport the iron that passes through the mucosal cells into the blood stream. This protein of mol. wt. 90,000 is orange in color because the iron complex that it forms is orange. The protein may have attached to it a maximum of two atoms of ferric iron. Normally this protein is only one-third saturated with iron and thus can take care of transporting more iron in the blood stream if that becomes necessary. If this protein were not present in the blood stream, iron would tend to add on to various proteins and bring about precipitation. We can readily see the undesirable effects of such iron when somewhat more than 10 mg. of ferrous iron is injected into man. This iron, in excess of that which will combine with the siderophilin, will cause nausea and

other symptoms of toxicity. It would not be surprising to find that such a protein also transports iron in the blood stream of the lower vertebrates, but as yet no one has looked for it. A protein, conalbumin, apparently forming an identical orange colored complex with iron is present in the egg white of chickens, where it may serve as a very dilute storage form of iron for the embryonic chick (Shade and Caroline, 1946).

In the battle for a place in the sun there has been literally much loss of blood. Those organisms could survive that could rather rapidly replace a partial blood loss. For example, what would happen in the case of an acute hemorrhage. Iron would be required to replace the loss. But there is relatively little available iron in food, and iron is absorbed in small quantities over long periods of time. Some mechanism for storing iron would provide a solution of this problem. Such a mechanism for storing iron is actually present in the body. This storage is in the form of a protein called ferritin, first discovered by Laufberger (1937). Ferritin contains as much as 23 per cent of its dry weight in the form of iron. There is enough of this ferritin iron in the human body to be used in the synthesis of approximately one-third of the body's hemoglobin if the need should arise.

Ferritin is present in highest concentrations in the spleen, liver and bone marrow, but may be found in lower concentrations in many other tissues. It is made up of a colorless protein, apoferritin, to which the iron becomes attached in the form of small clusters of ferric hydroxide micelles. A special attribute of this invention is that the protein apoferritin is synthesized in response to the iron brought to the tissue (Granick, 1946). Not only does the inorganic iron stimulate the tissue to manufacture this particular protein, but the iron then is deposited on this protein in a particular fashion. When iron is needed, say for hemoglobin synthesis, the cells will dissolve off the iron of the ferritin; at the same time the protein will be broken down, and its amino acids can be used for the synthesis of the proteins of the red blood cell.

Ferritin has been found in various mammals. Recently Tecce (1952) has found ferritin in chicken liver, and also in the liver of *Scyllium caniculata,* an elasmobranch. It would be interesting to examine various kinds of fishes to find out in what groups ferritin is present.

SUMMARY AND CONCLUSIONS

In summary, we have examined certain aspects of iron metabolism from an evolutionary point of view, and the following story is suggested:

Under the environmental conditions existing on the surface of this planet it is necessary to activate oxygen so that energy may be released, an energy that can be utilized for protoplasmic synthesis. This activation or catalysis of oxidation with oxygen was at first brought about by inorganic iron compounds. Later the inorganic iron compounds may have been replaced by simple organic iron compounds, then by iron protoporphyrin, and eventually by iron protoporphyrin proteins, that is, the cytochromes.

Since oxidative catalysis is a fundamental function it was never lost in evolving protoplasm. What evolved were iron catalysts of greater complexity, each one of great efficiency compared to inorganic iron and each one a specialist performing one task with high efficiency instead of the four tasks performed by inorganic iron in a mediocre way.

At a later stage of evolution, when the larger and more complex structures of vertebrates began to evolve, inventions (fig. 2) had to be perfected to supply sufficient O_2 to the iron oxidative catalysts of the cells, the cytochromes. One of these inventions was the iron-containing compound

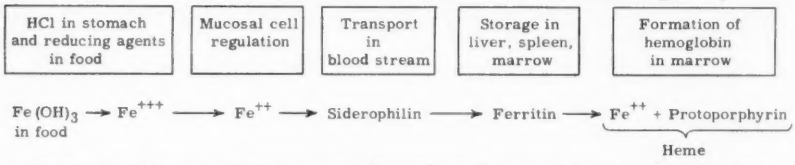


FIGURE 2. Scheme to indicate some inventions that were developed in the vertebrate to supply adequate amounts of iron for hemoglobin synthesis.

hemoglobin. For large animals to have an adequate O_2 transport, a large amount of hemoglobin was required. In terms of the amounts of iron needed for the oxidative enzymes common to all life, this was a biologically huge amount of iron, and it was necessary to absorb it in the ferrous form. Because of the autoxidizability of ferrous iron at body pH a new invention appeared in the craniata, an HCl-secreting cell in the stomach. The HCl dissolved the ferric hydroxide or loose ferric complexes contained in food; the food contained reducing substances that could bring about at acid pH the conversion of the ferric iron to the ferrous state; and the acid pH resulting from the HCl made possible the stabilization of ferrous iron by preventing it from being autoxidized back to the ferric state by the O_2 present.

To regulate the amount of iron in the body, an invention for regulation appeared in the mucosal cell of the intestinal tract, that is, at the port of entry rather than at the port of exit. Iron once entering the body never leaves it. The iron is used over and over again. The invention of the mucosal cell was such that only small amounts of iron normally entered, but in cases of hemorrhage or when body demands were great more iron would be permitted to enter through the mucosal cell into the blood stream. The iron entering the blood stream was transported to the various tissues of the body by means of another invention, a special protein called siderophilin.

To take care of sudden demands for more iron, as in case of acute hemorrhage, another invention appeared, namely, an iron protein, ferritin, which serves as a storage form of iron. The iron of this protein, stored previously in liver and spleen, could be rapidly made available for the demands of the bone marrow where the blood cells are synthesized.

I have presented this discussion on iron metabolism to illustrate how simple biological and chemical considerations may permit one to recon-

struct certain phases of evolution. It is hoped that more correlations and deductions of a similar type may be possible. Eventually, one may come to know of a kind of biology that existed when the earth was very young, and that may exist even today.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

OBSERVATIONS ON THE COMPLEMENTS OF OVA AND NESTING OF THE FOUR-TOED SALAMANDER IN VIRGINIA

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The four-toed salamander, *Hemidactylium scutatum* (Schlegel), has recently been shown to be abundant on the Virginia coastal plain (Wood, 1951). This abundance permits a comparison of the nesting characteristics of this species in a southern state with the excellent studies of this species in the northeast (Bishop, 1941) and mid-west (Blanchard, 1922, 1934, 1936; Blanchard and Blanchard, 1931).

A total of 224 nests of Hemidactylium eggs were examined in the field in late February, March, and early April of 1950 and 1951 in 12 of the 100 counties of Virginia, as follows: Appomattox, Charlotte, Charles City, Elizabeth City, Gloucester, James City, Lancaster, Mathews, Middlesex, Richmond, Warwick, and York. The first two of these counties are on the Piedmont plateau, and all others are on the coastal plain. Collections consisted of 144 nests containing over 16,000 eggs, and 248 attending specimens. The eggs in three of the nests were hatched to obtain a series of 82 recently emerged larvae. All other eggs and attending Hemidactylium were preserved within a few hours after collection. Each discrete egg group was isolated in an individual container in the field, together with all attending specimens. Great care was exercised in attempting to avoid the loss of eggs during collecting. After preservation the number of eggs in each nest was determined, and the attending specimens were measured for total length and snout-vent length. Every salamander was then dissected to verify its sex, and check its stomach contents. The ovaries and oviducts were examined in all females; numbers of large ova and their distribution were noted. Gravid females contained ova of two types, as follows: (a) Large yellowish deutoplasm-filled ova (2.0 to 3.0 mm. diam.); and (b) small whitish translucent ova (0.2 to 0.8 mm. diam.). The large ova were ready for deposition during the current egg-laying period, and the small ova comprised the ova complements of future seasons.

The numbers of eggs in discrete nests ranged from six to 868. Since no gravid female contained more than 80 large ova, it is likely that the great majority of nests containing more than 80 eggs result from the combination of part or all of the ova complements of two or more females. It is also likely that a number of the nests containing less than 80 eggs are also derived from more than one female. The counts of eggs in 141 nests are arrayed in the histograms in figure 1. Forty per cent (56) of the nests contain

80 or more eggs, and sixty per cent contain less than 80 eggs. A sharp unimodal peak and normal distribution curve in the latter series suggests that these nests may have been deposited by one female in each case; the marked central tendency in numbers of eggs includes those nests containing from 30 to 50.

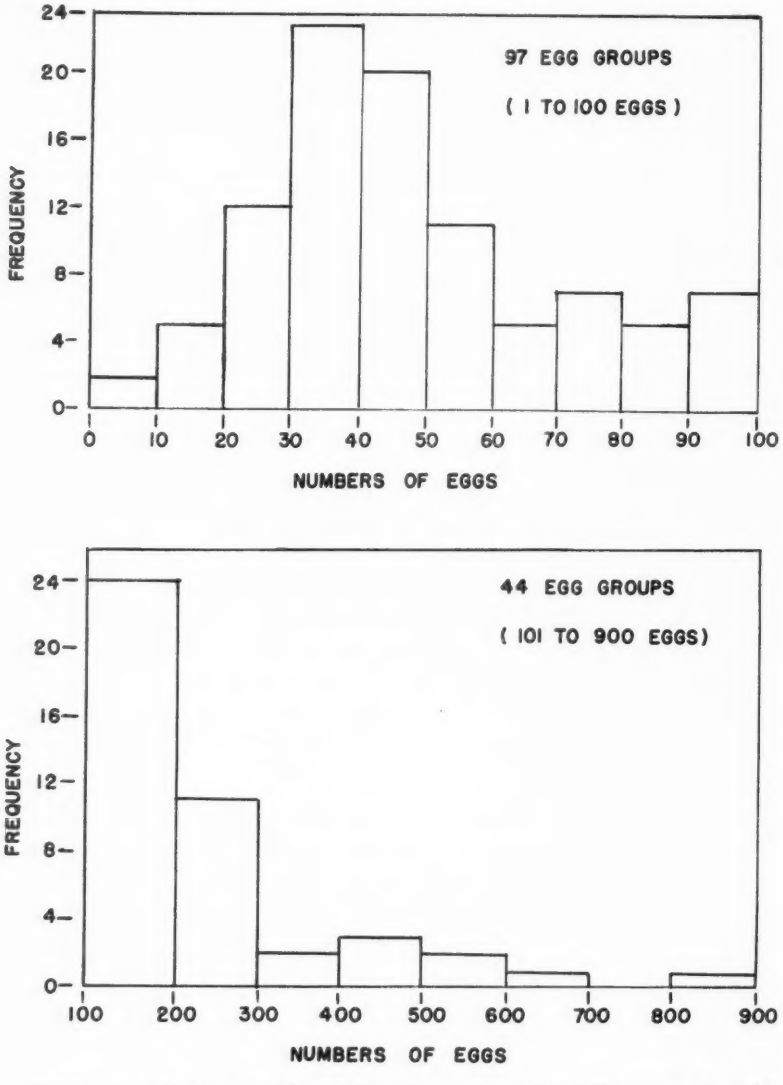

FIGURE 1. A histogram of the number of eggs counted in 141 nests of *Hemidactylium scutatum* (Schlegel) in Virginia.

In other areas lower limits have been established for the maximum number of eggs one Hemidactylium deposits in a nest. In the vicinity of Ithaca, New York, any nest containing more than 40 eggs is regarded as the product of more than one female (Gilbert, 1941). In a series of 47 nests examined in Pennsylvania, none were found to contain more than 50 eggs (Pawling, 1939). In the course of many years of field work in central New York Bishop (1941) reports finding only one nest of such size that it might have resulted from the product of more than one female, and he reports that the average number of eggs in a nest is about 50. Although the average nest in southern Michigan contains about 30 eggs, Blanchard (1934) reports nests of 120, 173, 247, 250, 320, 435, 487, 644, and 1,110 eggs. Current evidence points to predominantly solitary nesting habits in New York and Pennsylvania, and, in addition to solitary nesting, a considerable amount of gregarious nesting in Michigan and Virginia.

Virginia nests are frequently attended by transformed specimens. Dissections of the 248 specimens from nest sites substantiated Blanchard's (1922) evidence that only females attend nests. The attending specimens included 243 adult females, one adult male, two juvenile females, and two juvenile males.

Egg groups were attended by from one to four females during the first nine days of the egg-laying period (table 1), but in the three weeks following the two-week deposition interval, one of every four nests was found to be attended by no female (table 2); and several weeks later when the eggs were hatching, very few females could be found in the nest sites. Evidence of this departure of the females during the incubation period is reflected in the differences in the average number of eggs per female during egg-laying (table 1), and three weeks after this (table 2). By the mid-point in the incubation period, three of every five females in the Five Forks habitat, James City County, had deserted the nesting area. Nest desertion is reported not to occur in New York (Bishop, 1941), where females remain with the nest throughout the incubation period; and in every nest a female was found "guarding the eggs", even though the majority showed an advanced stage of development (Gilbert, 1941).

TABLE 1

NESTS COLLECTED DURING THE FIRST NINE DAYS OF EGG-LAYING

No. of females	No. of nests	Total no. of eggs	Av. no. of eggs per nest	Av. no. of eggs per female
1	23	1277	56	56
2	14	1354	97	48
3	7	921	132	44
4	2	939	468	117
Totals	46	4,011		

Average number of eggs per female: 50.2

TABLE 2

NESTS COLLECTED DURING THE THREE WEEKS FOLLOWING EGG-LAYING PERIOD

No. of females	No. of nests	Total no. of eggs	Av. no. of eggs per nest	Av. no. of eggs per female
0	23	3,201	140	....
1	57	4,747	83	83
2	13	2,522	194	97
3	3	698	233	79
Totals	96	11,168		

Average number of eggs per female: 121.0

DESERTION OF NESTS

Blanchard (1934) finds that in Michigan females usually attend their nests until hatching occurs. However, if the nests are closely associated with others, all but one of the females usually desert the nest, and "only occasionally will even two remain." This desertion of the large nests may occur very early in the egg-laying period in Virginia. In one nest of 695 eggs five females were found attending on March 4, 1951, a time when the nesting season was only nine days old. If we estimate an average deposition of 50 eggs per female, the nest must have been formed by the combined complements of 14 specimens, thus approximately nine had already deserted the nesting site.

No one has determined why females remain in the vicinities of their nests following egg-laying. Noble (1930) proposed the theory that female marbled salamanders, *Ambystoma opacum* (Gravenhorst), remained with their eggs after depositing them because of exhaustion. If this theory is used to explain the presence of Hemidactylium by their nests, it must be pointed out that this "state of exhaustion" appears to be quite transitory, and can be voluntarily terminated by the female. When the nest is exposed by a collector, this "state of exhaustion" is promptly transformed to a "state of activity." If the disturbance to the nest is minor, the female retreats slowly, frequently backing away into the surrounding debris and moss. If the disturbance is major, the female retreats rapidly, either turning and dashing with undulatory movements into surrounding debris, or dropping into the adjacent water. Once in the water, the female swims for a few inches beneath the surface, and stops and creeps back up to the surface after encountering roots and vegetation. The first behavior pattern appears to be a negative response to light, and the second is a response to alarm. At no time has the author noted any aggressive behavior on the part of the female, but Gilbert (1941) reports observing females "guarding" their nests in New York State. If females remain beside their nests because of exhaustion, it is probable that they would tend to depart following recuperation. It would be a striking coincidence if the recuperation period invariably paralleled the incubation period of the eggs.

During the egg-laying period many nests were found to be attended only by gravid females, the post-partum females having already departed. The males and juveniles near egg groups appear to be the result of accidental distribution; no other case of adult males attending nests have been found in the literature.

The hunger drive is probably one incentive motivating the females in their desertion of the nests. Field observations in Virginia have shown that while Hemidactylium are attending the nests, they remain near the eggs night and day. Dissections of the stomachs of 243 attending females revealed no food present. The relatively slow digestive rate in salamanders makes this strong evidence that females do not wander off and feed during the period of their attending nests.

The position of the attending female in relation to the egg mass has been described by Bishop (1920) as follows: "In every instance the female was found either partly coiled upon the eggs or about them." In Virginia greater variation in this relationship has been noted, but in the majority of nests in the habitats that Bishop describes, females were found lying beneath the eggs. Females were only found coiled about eggs or lying on top of them in habitats containing natural crevices so small that there was no room beneath the eggs for the female. This type of habitat was found under loose bark, and in nests attached to the rhizoids of Cirriphyllum, in shallow depressions on eroded brook banks. The eggs in the latter habitat were frequently in contact with the rhizoids above, and the earth below, and no natural crevices remained under them.

EGG MORTALITY

Dead eggs were noted in many nests, and proportionately greater numbers of these were noted late in the incubation period. Egg mortalities were more frequent in Sphagnum than in mosses of other genera. Dead eggs were more common in the crowded aggregations of large nests than in small, loosely placed egg groups. Dead eggs are distinguished by their opaque appearance, as contrasted with the translucent or transparent envelopes of living embryos. The causes of these mortalities are undetermined, but seem to be correlated with crowding. Bishop (1920) reports that excessive moisture is lethal to laboratory-incubated eggs, and Green (1941) cites a case of natural mortality in Hemidactylium eggs in a wet habitat. Since the author submerged several groups of eggs in the laboratory, and permitted these eggs to stay under water through the incubation period, excessive moisture apparently is not a lethal factor. These submerged nests produced normal larvae in the normal incubation period without any egg mortality involved.

SIZE OF NEST ATTENDANTS

Blanchard and Blanchard (1931) found that the mean total lengths of Hemidactylium attending nests in southern Michigan varied from locality to locality, and they reported three means for discrete groups, as follows: 74.1 mm., 74.7 mm., and 83.7 mm. Bishop (1941) noted that in central New

York the mean total length of attending females is 70.9 mm. No attempt was made in Virginia to detect variations associated with localities; the mean total length of 204 adult females attending nests is 78.4 ± 2.9 mm., with a range from 60.5 to 94.5 mm. Since there is no certain way of determining whether the specimens have complete original or partially regenerated tails, an error is introduced into total length data. Of 39 Virginia specimens with incomplete or missing tails, 38 had lost theirs at the basal constriction. When regeneration occurs from the basal constriction it is difficult to determine from pigmentation and general form whether a tail is original or not. Not-apparent regeneration must play a part in the extensive variation in tail lengths in any size-group, as noted by Blanchard and Blanchard (1931), but the range in total lengths has been included because all other published reports provide these figures. Greater reliability can be placed on snout-vent measurements for establishing regional differences or smiliarities.

The snout-vent lengths of 243 adult females, four juveniles, and 82 recently emerged larvae (fig. 2) show three discrete mutually exclusive groups of measurements. The distribution curves of adult and larval snout-vent lengths are unimodal, with no year-classes evident in the former. Blanchard and Blanchard (1931) provided excellent data on growth of Hemidactylium, which show that the four juveniles are yearlings. The age of recently emerged larvae is from a few hours to a week; yearlings, from 10 to 12 months; and the age of the adults is less certain. Blanchard and Blanchard (1931) interpret their data to find that Hemidactylium attains fecundity in southern Michigan during its third year of life. In Virginia growth from the mean snout-vent length of larvae to yearlings involves an increase of 150 per cent over larval length, and considerably less increase in total length due to ontogenetic differences in rate of tail growth and rate of snout-vent length increase. Growth from yearlings to adults involves an increase of only 60 per cent from mean yearling snout-vent length to mean adult snout-vent length. From the standpoint of rate of growth it is evident that adult size can be attained in 20 to 22 months. It is possible that attainment of

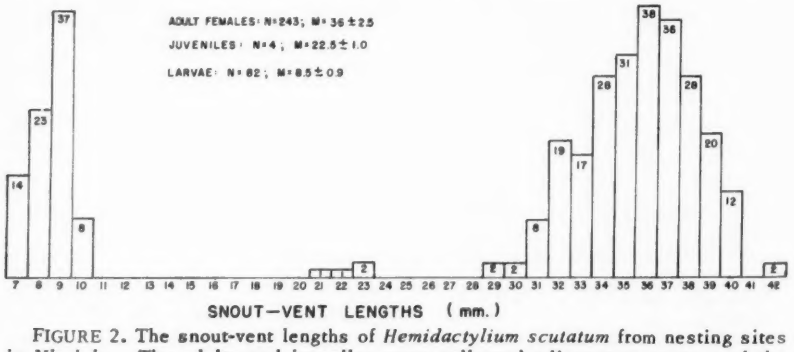

FIGURE 2. The snout-vent lengths of *Hemidactylium scutatum* from nesting sites in Virginia. The adults and juveniles were collected adjacent to nests, and the larvae were hatched from egg groups and preserved within a week of emergence.

adult size precedes attainment of sexual maturity by a year, and that an adult-sized group of immature females of age 20 to 22 months was absent from the breeding grounds, while the egg-laying females ranged from 30 to 32 months of age, or older.

NUMBERS OF EGGS

Large ova were counted in 58 gravid females (fig. 3, above). In 32 of these specimens all of the large ova were found in the ovaries, and the oviducts were undistended; in the other 26 specimens, in addition to ovarian eggs, from one to ten oviducal eggs were noted, and the oviducts were distended and tortuous. The former group consisted of females preserved prior to egg-laying; the latter group contained specimens with partially spent ovaries, and their total remaining large ova were not interpreted as evidence of their original complements of ova. A line, based on females with full complements of ova, was plotted to fit these data by the method of least squares; a strong and highly significant lineal correlation was evident between the snout-vent length of a female and her complement of large ova. Blanchard (1936), relating the lengths of 217 southern Michigan females to their complements of ova, found no significant correlation. Possibly introduction of variables by his use of total lengths, or inclusion of some partially spawned females, may have concealed the relationship.

Other differences between the Michigan and Virginia series include the number of large ova in gravid females; Virginia specimens had from 29 to 80; Michigan specimens, six to 46 (Blanchard, 1936). The magnitude of this difference suggests that different physiological races may be involved, a question raised first by Blanchard in regard to the apparent differences between New York and Michigan Hemidactylium.

Blanchard (1936) selected 50 nests of which each was attended by one female and contained a complement of eggs not in excess of the potential production of one Michigan specimen. He compared the average number of eggs in these nests with the average of large ova complements in gravid females, and found that these values were approximately the same. His interpretation was that normally all eggs are laid in one nest, and that this nest is attended by the female laying the eggs. Since a lineal relationship has been found between the snout-vent length of a female and her egg complement, it is possible to test any similarity in nest and ovarian egg complements by introducing a factor common to both, the snout-vent length of the female. The snout-vent lengths of each female attending a Virginia nest containing less than 80 eggs (57 nests) are plotted against the egg counts in figure 3, below. A weak correlation of no statistical significance was obtained. A line was fitted to these data by the method of least squares. The dissimilarity in the slopes of the lines in figure 3, upper and lower, demonstrates the lack of close relationship between the large complements of ova of females and the number of eggs in nests they attend. This is evidence that females do not deposit all of their eggs in one nest, and attend it.

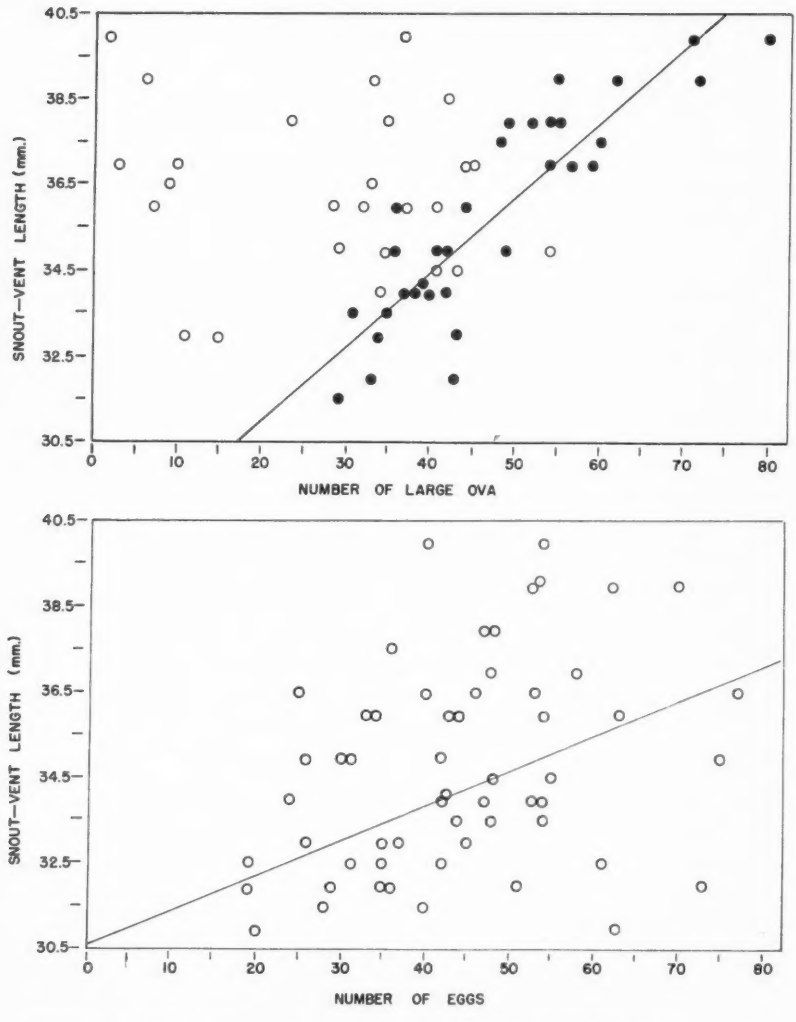

FIGURE 3. (Above) The snout-vent lengths of 58 gravid *Hemidactylium scutatum* (Schlegel) plotted against their large ova complements; dots denote females (32) in which large ova were all in the ovaries, and circles (28) represent females in which one or more ova have entered the oviducts. The line, based on females having ovarian ova only, was determined by the method of least squares.
(Below) The snout-vent lengths of 57 attending females plotted against the number of eggs in their nests; the line was fitted to these data by the method of least squares.

The scatter of the points in figure 3, below, suggests that many points below the line represent small nests deposited by two or more females, and some points above the line represent partial deposition of the complement

of one female, or destruction or loss of eggs. The number of small females attending large egg groups can only be accounted for in the former way. These combined nests are so small that it seems possible they contain less than the full egg complements of two females, suggesting that one female, interrupted in the act of egg-laying, departs from the nest after depositing some of her eggs, and the intruding female adds her complement of ova to the group.

The behavior dynamics of Hemidactylium in nesting sites are little understood, but it has been mentioned that females readily desert the sites of gregarious nesting. That females may normally deposit less than their full complement of ova in one nest is indicated by the 12 nests attended by females that contained fewer eggs than the minimum number noted in gravid Virginia specimens. Undoubtedly some eggs are lost during collecting; losses prior to collecting are similarly unknown. Whether or not females usually successfully void their ovaries of all large ova during egg-laying is of importance in interpreting the points above the line in figure 3 (below). Blanchard (1936) states that all large ova are normally deposited by Michigan specimens, though he cites one exception. This is not true in Virginia. In 186 dissections of the ovaries of spawned females it was noted that more than half of the specimens contained resorbed or partially resorbed large ova. These appeared as aggregations of melanin, occasionally accompanied by yolk masses. No characteristic distribution of residual large ova was noted, their scatter through the ovaries apparently being a random pattern. Retained resorbed ova did not number in excess of ten in any specimen examined. It is interesting to note that in *Cryptobranchus alleganiensis* Daudin, the failure to void the ovaries of all large ova at the time of egg-laying has been attributed to the inefficiency of the mechanism for transfer of the ova into the funnels of the oviducts (Smith, 1916).

SUMMARY

1. Nests of Virginia *Hemidactylium scutatum* (Schlegel) contained from six to 868 eggs.

2. Of 248 specimens attending nests, 243 were adult females, two were juvenile females, one was an adult male, and two were juvenile males.

3. Forty per cent of 141 nests examined contained the eggs of two or more females.

4. Three of every five females had deserted the nesting site by the time the incubation period was only half over; few were found in nesting areas when the larvae were emerging.

5. Females will desert gregarious nests before the egg-laying period has terminated.

6. Exhaustion of the females may be the cause of their attending nest sites immediately after egg-deposition; the hunger drive and recuperation may account for their later desertion.

7. The position of the female in relation to the egg group depends upon the available space in the nesting site.

8. Dead eggs are found in many nests. Excessive moisture may be a contributory cause, but is not the direct cause of this mortality.

9. The total lengths of adult females attending Virginia nests ranged from 60.5 to 94.5 mm., with a mean of 78.4 ± 2.9 mm.

10. In Virginia, adult size can be attained in from 20 to 22 months; this may precede the attainment of sexual maturity by a year.

11. A strong, significant lineal correlation exists between the snout-vent length of gravid females and their large ova complements; Virginia Hemidactylium contained from 29 to 80 large ova prior to egg-laying.

12. There is no strong correlation between the snout-vent lengths of females and the numbers of eggs they attend.

13. *Hemidactylium* do not successfully void their ovaries of all large ova during egg-laying. This fact, plus nesting behavior, accounts for the lack of close relationship between the number of large ova a female produces, and the number of eggs she is found attending in the field.

ACKNOWLEDGEMENT

This paper is adapted from part of a thesis submitted to the faculty of the College of William and Mary in 1951.

I wish to thank Dr. J. T. Baldwin, Jr., Dr. R. P. Ash, Dr. O. F. Schuette, Jr., Dr. E. R. Sohns, and Dr. J. L. McHugh for critical reading of the manuscript and many suggestions. I am indebted to the following field assistants: O. K. Goodwin, I. L. Fuller, J. B. MacArthur, C. D. Ronemous, W. T. Allen, L. T. Duval, and S. S. Forest.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

COMPOSITION OF WILD POPULATIONS IN THE LYCAENID BUTTERFLY *NEOZEPHYRUS TAXILA**

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In many Lycaenid butterflies of the genus Zephyrus and its allies the females are polymorphic. As far as the writer knows, this polymorphism has never been genetically analyzed. *Neozephyrus taxila* Bremer is a typical example of such butterflies. Its subspecies, *N. t. japonicus,* is endemic in the main island (Honshū) of Japan, and occurs rather commonly in its mountainous districts. In Hokkaidō it is represented by a distinctly smaller subspecies, *N. t. taxila.* The larvae of both subspecies feed on the leaves of Alnus spp. This species, as throughout the same and allied genera, has only one brood a year, and the butterflies appear in summer, from July to September. The eggs are laid singly on young branches of the food plant, and do not hatch until the spring.

This butterfly shows distinct sexual difference in color and marking. In the male the upper side is metallic green all over, except for the black distal margin. In the female the ground color of the upper side of the fore wing is either universally dark brown, or with markings. Thus, the female has the four color types: (1) without marking, (2) with an orange marking in both the third and fourth cells, (3) with a bluish suffusion occupying the whole of the Ib and discoidal cells, and (4) with both the orange markings and blue suffusion (fig. 1). The nomenclature of these four types, according to Esaki (1935), is as follows:

Type	Subspecies *japonicus* Murray	Subspecies *taxila* Bremer
1 (O)	*japonicus* Murray	*taxila* Bremer
2 (A)	*leechi* Esaki	*maculatus* Rühl
3 (B)	*fasciatus* Janson	*quercus* Kardakoff
4 (AB)	*pryeri* Esaki	*regina* Butler

In the parentheses are presented the symbols which many Japanese collectors are accustomed to use.

A few years ago, the writer conceived the idea that these four types might be due to a set of triple-allelic or triple "semiallelic" genes (Komai, 1950), namely, a recessive gene for the plain color (O) and two dominant genes, one for the A marking and the other for the B marking. However, owing to the great difficulty in getting eggs from this butterfly in captivity, no crucial breeding data for testing this hypothesis have been available.

*Contribution No. 33 from National Institute of Genetics, Japan.

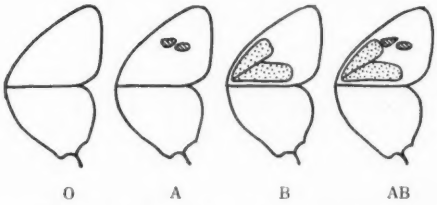

FIGURE 1. Diagrammatic figures showing the four color types among the females of *Neozephyrus taxila* (after Esaki and Shirōzu). Hatched area corresponds with orange markings, stippled area with bluish markings.

But the writer has been able to obtain some data concerning the composition of some population samples mostly of the subspecies *japonicus* supplied by his colleagues. These data are presented in table 1, and the localities are shown in the map in figure 2. Of the altogether thirteen samples, No. 13 is of the subspecies *taxila*, while the rest are of the subspecies *japonicus*. A few samples, e.g. Nos. 7 and 8, were collected in a period extending over several years, while most of them were obtained in one period of the emergence of this butterfly. The specimens were collected in deciduous woods less than a few kilometers in extension, which seemed to be nearly uniform throughout ecologically, at least for the life of this butterfly.

TABLE 1

THE SOURCE AND COMPOSITION OF THE SAMPLES

Sample no.	Locality	Collector	$\bar{O}$	$\bar{A}$	$\bar{B}$	$\bar{AB}$	N
1	Mt. Asama (1922-'23)	S. Kobayasi	413 (0.570)	172 (0.238)	83 (0.115)	56 (0.077)	724 (1.000)
2	Mt. Asama (1929)	T. Niimura	45 (0.454)	22 (0.222)	27 (0.273)	5 (0.051)	99 (1.000)
3	Oiwake (1950)	T. Niimura	28 (0.596)	10 (0.213)	8 (0.170)	1 (0.021)	47 (1.000)
4	Rondenike (1950-'51)	K. Teruyama, et al.	84 (0.332)	40 (0.158)	100 (0.395)	29 (0.115)	253 (1.000)
5	Tokiwa (1951)	A. Hosono	25 (0.316)	11 (0.139)	23 (0.291)	20 (0.253)	79 (0.999)
6	Suwa (1930)	T. Niimura	41 (0.398)	28 (0.272)	17 (0.165)	17 (0.165)	103 (1.000)
7	Syakuzii (1929-'37)	K. Hayashi	65 (0.650)	11 (0.110)	16 (0.160)	8 (0.080)	100 (1.000)
8	Ibaraki (1935-'40)	S. Murayama	12 (0.169)	0 (0.000)	58 (0.817)	1 (0.014)	71 (1.000)
9	Sendai (1951)	Y. Ono	1 (0.036)	4 (0.143)	21 (0.750)	2 (0.071)	28 (1.000)
10	Rondenike (1952)	K. Teruyama	16 (0.254)	14 (0.222)	24 (0.381)	9 (0.143)	63 (1.000)
11	Kosimizuhara (1952)	K. Kobayasi	29 (0.276)	10 (0.095)	40 (0.381)	26 (0.248)	105 (1.000)
12	Tukude, Aiti (1951)	A. Takahasi, et al.	26 (0.491)	18 (0.340)	6 (0.113)	3 (0.057)	53 (1.001)
13	Onuma (1952)	K. Akino	3 (0.021)	3 (0.021)	105 (0.734)	32 (0.224)	143 (1.000)

FIGURE 2. Map of northern half of Honshū with the southern-most part of Hokkaidō indicating the localities where the samples were collected (×).

It was sometimes difficult to classify the specimens, especially worn ones. The specimens which had only a few orange or blue scales on the dark-brown background were classified as orange (A) or blue (B) ones.

From the data presented in table 1, the relative frequency of the genes for A, B and O types (p, q and r respectively) has been computed accord-

ing to the formulae familiar to blood-group specialists:

$$p = 1 - \sqrt{\overline{B} + \overline{O}},$$

$$q = 1 - \sqrt{\overline{A} + \overline{O}},$$

$$r = \sqrt{\overline{O}}.$$

In table 2 the results of such computations are shown. Of the thirteen samples, no. 9 is very small, and will be excluded from further consideration. Nos. 2, 3, 4, 8, 10, 12 and 13 accord well with the view given above which assumes a set of triple-allelic or triple-semiallelic genes as the basis of this polymorphism. This is shown by the value D falling within

TABLE 2

THE VALUES OF p, q AND r AND THE STANDARD ERROR OF THE DIFFERENCE BETWEEN THE UNIT AND THE SUM OF p, q, r (σD) FOR EACH SAMPLE

Sample no.	p	q	r	$p+q+r$	D	σD
1	0.172	0.101	0.755	1.028	−0.028	0.004
2	0.147	0.178	0.674	0.999	+0.001	0.013
3	0.125	0.100	0.772	0.997	+0.003	0.013
4	0.147	0.300	0.576	1.023	−0.023	0.013
5	0.221	0.325	0.562	1.108	−0.108	0.029
6	0.250	0.182	0.631	1.063	−0.063	0.018
7	0.100	0.128	0.806	1.034	−0.034	0.009
8	0.007	0.589	0.411	1.007	−0.007	0.008
9	0.114	0.578	0.187	0.879	+0.121	0.056
10	0.203	0.310	0.504	1.017	−0.017	0.030
11	0.189	0.391	0.525	1.105	−0.105	0.027
12	0.223	0.088	0.701	1.012	−0.012	0.016
13	0.131	0.795	0.145	1.071	−0.071	0.045

the limit of twice the corresponding σD calculated according to the formula: $D = \frac{1}{\sqrt{N}} \sqrt{\frac{pq}{2(1-p)(1-q)}}$ (Bernstein, 1930a). The similarity of the values

TABLE 3

THE VALUES $2pq$ AND $2\sqrt{\overline{A}}\sqrt{\overline{B}}$ COMPARED WITH $\overline{AB}$ FOR EACH SAMPLE

Sample no.	$2pq$	$\overline{AB}$	$2\sqrt{\overline{A}}\sqrt{\overline{B}}$
1	0.035	0.077	0.332
2	0.052	0.051	0.492
3	0.025	0.021	0.381
4	0.088	0.115	0.499
5	0.143	0.253	0.403
6	0.091	0.165	0.424
7	0.026	0.080	0.266
8	0.008	0.014	....
9	0.132	0.071	0.655
10	0.126	0.143	0.581
11	0.148	0.248	0.380
12	0.039	0.057	0.392
13	0.208	0.224	0.248

$2pq$ and the corresponding $\overline{AB}$ in all these samples also holds with this view (table 3).

In samples nos. 1, 5, 6, 7 and 11, however, the sum of p, q and r significantly exceeds the unit, and D is considerably larger than twice the corresponding σD in all of them; there is also rather great disagreement between $2pq$ and the corresponding $\overline{AB}$.

TABLE 4

CHI-SQUARE TESTS OF THE DIFFERENCES BETWEEN THE OBSERVED AND EXPECTED NUMBERS OF THE FOUR TYPES IN SAMPLES NOS. 1, 5, 6, 7 AND 11

Sample		$\overline{O}$	$\overline{A}$	$\overline{B}$	$\overline{AB}$	Total	P
1	Observed	413	172	83	56	724	
	Expected	386.367	200.524	112.604	24.506	"	
	Difference	+26.633	−28.524	−29.604	+31.494		
	Dif.²/Exp.	1.836	4.057	7.783	40.474	$\chi^2 = 54.15$	< 0.001
5	Observed	25	11	23	20	79	
	Expected	18.277	19.618	31.151	9.954	"	
	Difference	+6.723	−8.618	−8.151	+10.046		
	Dif.²/Exp.	2.473	3.786	2.133	10.149	$\chi^2 = 18.541$	< 0.001
6	Observed	41	28	17	17	103	
	Expected	34.926	35.030	24.283	8.761	"	
	Difference	+6.074	−7.030	−7.283	+8.239		
	Dif.²/Exp.	1.057	1.410	2.184	7.748	$\chi^2 = 12.400$	< 0.001
7	Observed	65	11	16	8	100	
	Expected	61.623	14.765	19.738	3.874	"	
	Difference	+3.377	−3.765	−3.738	+4.126		
	Dif.²/Exp.	0.185	0.960	0.008	4.394	$\chi^2 = 6.247$	0.01–0.02
11	Observed	29	10	40	26	105	
	Expected	21.357	20.318	49.417	13.908	"	
	Difference	+7.643	−10.318	−9.417	+12.092		
	Dif.²/Exp.	2.735	5.239	1.795	10.513	$\chi^2 = 20.282$	< 0.001

In table 4 are presented the results of chi-square tests to examine more crucially the significance of the differences between the observed and expected values in each class in the samples, nos. 1, 5, 6, 7 and 11, where the expected values were found on the basis of the adjusted p, q, r values (p', q', r') according to the following formulae:

$$p' = \left(1 - \sqrt{\overline{O} + \overline{B}}\right)(1 + D/2),$$

$$q' = \left(1 - \sqrt{\overline{O} + \overline{A}}\right)(1 + D/2),$$

$$r' = \left(\sqrt{\overline{O}} + D/2\right)(1 + D/2). \qquad \text{(Bernstein, 1930b).}$$

It is uniformly shown in these samples that the observed values significantly exceed the expected values in the classes $\overline{O}$ and $\overline{AB}$, whereas the reverse is true in the classes $\overline{A}$ and $\overline{B}$. This suggests that the heterozygote AB has some selective advantage over the homozygotes AA and BB which are included in the class $\overline{A}$ and $\overline{B}$ respectively, much as in other polymor-

phic butterflies (Fisher, 1927; Ford, 1937, 1940, 1946), as well as in other polymorphic animals—ground locusts, Lebistes and Cepaea (Fisher, 1930, 1939) and *Drosophila polymorpha* (da Cunha, 1949).

DISCUSSION

The facts given above, though deficient in some important points, notably breeding results, seem to support the view that the four different color types found among the females of this butterfly are due to a set of triple-allelic or triple-semi-allelic genes. If this view is wrong, we should postulate the mutual independence of the genes for the A and B markings, namely, their presence either in different chromosomes or at different loci of the same chromosome. Then the following relation should hold, under the condition of the panmictic mating and the absence of selection: $\overline{A} \times \overline{B} = \overline{O} \times \overline{AB}$ (Bernstein, 1930a).

TABLE 5

COMPARISON BETWEEN $\overline{A} \times \overline{B}$ AND $\overline{O} \times \overline{AB}$ FOR EACH SAMPLE

Sample no.	$\overline{A} \times \overline{B}$	$\overline{O} \times \overline{AB}$
1	0.027	0.044
2	0.061	0.023
3	0.036	0.013
4	0.062	0.038
5	0.040	0.080
6	0.045	0.066
7	0.018	0.052
8	0.000	0.024
9	0.107	0.003
10	0.085	0.036
11	0.036	0.068
12	0.038	0.028
13	0.015	0.005

These values computed for all the samples are shown in table 5. The discord between the corresponding values in all the samples seems to be too great to be attributed to mere sampling error.

It might also be suspected that the A and B markings are due to recessive genes instead of dominant genes. If so, $\sqrt{\overline{A}}$ and $\sqrt{\overline{B}}$ should represent respectively the incidence of the gene for A marking and that of the gene for B marking, and $\overline{AB}$ should be equal to twice the product of these values: $\overline{AB} = 2(\sqrt{\overline{A}} \times \sqrt{\overline{B}})$. However, this is far from the case, as shown in table 3.

As far as the writer knows, no case exactly comparable to the present one has been demonstrated in butterflies or moths. There are certainly several cases among butterflies where the female is dimorphic (e.g. Colias spp., *Argynnis paphia*), or polymorphic (e.g. *Papilio polytes, P. memnon, P. dardanus*). This dimorphism has been shown to be governed by a single pair of Mendelian genes (Colias—Gerould, 1923; Ford, 1937; Hovanitz, 1944; Komai and Aé in press; *Argynnis paphia*—Goldschmidt and Fischer, 1922); while the polymorphism in the Papilios has been interpreted on the

basis of independent pairs of genes (*P. polytes*—Freyer, 1913; *P. memnon* —Jacobson, 1909; de Meijère, 1910). The case in *Neozephyrus taxila* here described differs from these previous cases in that we are dealing with polymorphism which is apparently due to a set of triple-allelic or triple-semiallelic genes.

It might be pointed out that the orange color in this butterfly found in the types A and AB is undoubtedly a pigmentary color, whereas the bluish color found in the types B and AB is a structural color. So the real allelism of the genes producing the colors of such different nature seems a priori rather improbable. Rather, the semiallelism of these genes as seen in the ground locusts and Lebistes is more probable.

The polymorphism in *Neozephyrus taxila* differs from that in the Papilios in that no mimetism comes into question. Of the four types, O is most somber, and undoubtedly best fitted for cryptic purpose. The orange or blue marking found in other types is more conspicuous, but it may confer some advantage on the individual possessing it. The dominant genes for these colors, however, probably become disadvantageous in the homozygous state, lowering the fertility or vitality of the possessor, as has been demonstrated in those butterflies (*P. polytes*, Freyer, 1913; *Colias philodice*, Gerould, 1923; Hovanitz, 1944, *Argynnis paphia*, Goldschmidt and Fischer, 1922), ground locusts (Fisher, 1930, 1939), and more recently in *Drosophila polymorpha* (da Cunha, 1949). This is suggested by the relative excess of the heterozygotic class $\overline{AB}$ and the recessive class $\overline{O}$ and the deficiency of the classes $\overline{A}$ and $\overline{B}$ in several population samples.

The available data are insufficient for demonstrating the existence of a geographical gradient in the incidence of the genes for these marking types, such as found in the variable lady-beetle Harmonia (Komai, Chino and Hosino, 1950). However, there is apparently a tendency for the incidence of the gene for B being higher in populations of this butterfly found in Hokkaidō and in the northern districts of Honshū, than in the more southern districts.

As stated in the beginning of this paper, there are some other species of the genus Neozephyrus and its allied genus Favonius occurring in Japan, which have polymorphic females like *N. taxila*. These species are less common than *taxila*, and our knowledge of the polymorphism in these species is quite limited. Still, it is more than probable, that the polymorphism in these species has the same genetic background as that in *N. taxila*. Such species according to Esaki (1935–1938), Esaki and Shirōzu (1950) and Hayashi (1951) are tabulated in the following list:

Species	Types among females
Neozephyrus aurorius Oberthür	O, A, B, AB
N. hisamatsuanus Nagami et Ishiga	B, AB
N. ataxus kirishimaensis Okajima	B, AB
Favonius ultramarinus Fixsen	O, A, B, AB
F. saphirinus Staudinger	O, A, B.

SUMMARY

1. In the Lycaenid butterfly, *Neozephyrus taxila* Bremer the female is polymorphic, and comprises the following four types: (1) plain dark-brown, (2) with orange markings, (3) with bluish markings, and (4) with both the orange and bluish markings. Although no breeding data are available, this polymorphism is apparently due to a set of triple-allelic or triple-semi-allelic genes, namely, a recessive gene for the plain color, a dominant gene for the orange marking and another dominant gene for the bluish marking.

2. Thirteen population samples from various localities in Japan include these types in different proportions. The relative frequency of these genes in these samples has been computed under the assumption given above. It has been revealed that, excepting one very small sample, seven out of the remaining twelve show that the observed incidences of the genes for orange marking *(p)*, bluish marking *(q)* and plain color *(r)* accord with the corresponding incidences expected on the basis of the above assumption. For the other five samples, there are discrepancies between the expected and observed values which exceed twice the standard errors. This is because the classes that include homozygotes of these dominant genes are relatively deficient, while the heterozygous (double-dominant) class and the recessive class are relatively in excess.

3. The polymorphism found in this butterfly seems to be comparable with the polymorphism in other butterflies, as well as in other animals previously studied, in that the dominants have some selective disadvantage in the homozygous state and some advantage in the heterozygous state at least under some conditions, and thereby the relative frequency of the dominant and recessive types is kept in the equilibrium state.

4. It is highly improbable that these four types are due to two sets of independent dominant genes, nor is it likely that they are due to a set of triple-allelic genes of which those for the markings are recessive to that for the plain color.

5. The names of other species in the same and allied genera showing similar polymorphism in females are listed.

The writer is indebted to Dr. E. B. Ford for valuable criticism.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

THE ORIENTATION OF CLADOCERA TO POLARIZED LIGHT*

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Von Frisch (1948) has shown that bees orient by means of a light compass reaction to polarized light. Waterman (1950) has shown that the compound eye of Limulus is capable of detecting polarized light. It is not only unlikely that the ability to detect polarized light is confined to these two groups, but, as will be shown, it is inescapable in a host of organisms. The particular interest in the Cladocera concerns the difference of their response from that of the bees.

MATERIALS AND METHODS

The following genera and species of Cladocera were tested: *Sida crystallina* (Müller), *Daphnia magna* (Straus), *Daphnia* sp., *Simocephalus vetulus* (Müller), *Simocephalus serrulatus* (Koch), *Ceriodaphnia reticulata* (Jurine), *Moina affinis* (Birge), *Bosmina obtusirostris* (Sars), *Kurzia latissima* (Kurz), *Chydorus globosus* (Baird), and *Leptodora kindtii* (Focke). They were used within a day after their collection from local ponds and lakes. If kept for longer periods of time they were fed yeast, except *Leptodora kindtii*, which was fed smaller Cladocera.

Experiments were conducted in one-gallon, rectangular, glass-sided aquaria filled with pond water made slightly turbid with suspended yeast. It is important to have the bottom and sides of the aquaria reflect as little light as possible. Adequate absorption may be secured by lining the aquaria with black wax.

The light source was a Spencer microscope lamp focused through a polaroid disc to give a parallel beam of light polarizable in any desired plane.

Upwards of 100 animals of the same species were placed in an aquarium and illuminated from above or from one side with polarized light in order to determine their response. No effort was made to estimate the number of animals responding, since in all experiments reported here over seventy-five per cent of the population responded simultaneously and similarly to any given situation.

RESULTS

All twelve species of Cladocera tested in polarized light responded by swimming back and forth in the light at right angles to the plane of vibration.

Under a vertical beam of light the population swims back and forth in the light at right angles to the plane of vibration. If the plane of vibration is

*Supported in part by a grant from the Horace H. Rackham School of Graduate Studies, University of Michigan.

rotated 90 degrees the population promptly responds by changing course 90 degrees. If the polaroid disc is rotated slowly through 360 degrees the population can be induced to swim in a circle.

In a horizontal beam of polarized light vibrating in the horizontal plane, the animals swim upright, which is at right angles to the plane of vibration. If the polaroid is turned so that the plane of vibration is vertical, the animals maintain their orientation by swimming on their sides. This reaction introduces considerable yaw into their course toward the light source and the result is a tacking maneuver.

In one collection of Cladocera there appeared about 100 water mites and 25 swimming caddisfly larvae which responded to a vertical beam of polarized light in the same way the Cladocera did. They were not tested in a horizontal beam of light.

In other collections there appeared mosquito larvae which also orient to polarized light but in a different manner. While they may glide in any direction under polarized light, they will turn when the plane of vibration is rotated. This response compares with that described for the bees.

The responses for Cladocera reported above occurred in summer animals at 25° to 28° C. They were still present in daphnids conditioned for five weeks at 4° C.

The results of preliminary examinations of the eyes and ocelli of several live *Daphnia magna* with a polarizing microscope in an effort to establish the presence of birefringent material which could serve as a detector of polarized light were negative. Similar results were obtained from a preliminary examination of fixed and stained material.

Oscillation of the cladoceran eye has been studied by Hess (1913). Further experiments in this laboratory suggest that this oscillation is a scanning mechanism for the detection of simple intensity changes or of polarized light or both.

DISCUSSION

The cladoceran eye, along with the water mite's ocellus, offers a simple mechanism for the detection of polarized light which may be extended to the compound eye of the Arthropoda. The principle is discussed briefly by Boehm (1940) as a doubtful explanation of the Haidinger's brush phenomenon in the human eye. (See Robert Wood, Physical Optics, p. 343. The Macmillan Co., N. Y.)

Polarization of light can be brought about by reflection and refraction of a beam of light passing from a less dense to a more dense medium. The extraordinary ray (vibrating parallel to the reflecting surface) tends to be reflected while the ordinary ray tends to be transmitted. This principle is applicable to the detection of polarized light by arthropods.

The ocellus of the water mite is a highly refractile sphere protruding laterally from a shallow pigment cup. While this eye cannot look up, the mite is obviously sensitive to dorsal polarized light. Such detection is plausible if the light beam, entering the outer portion of the sphere from

above, is reflected from the lower internal surface of the lens into the pigment cup. Since the ordinary ray will pass on through the lens the reflected light will consist largely of the extraordinary ray.

Such a mechanism is automatically sensitive to polarized light from above. It will record different intensities when the mite faces in different directions. If the long axis of the eye is parallel to the plane of vibration of the polarized light, no light can be reflected to the sensitive spot, while if the long axis of the eye is at right angles to the plane of vibration, an abundance of light may be reflected into the pigment cup.

The mite has two of these laterally-projecting ocelli, facing somewhat forward and at about 90 degrees to each other. The behavior of the mite, weaving to one side and the other as it swims in polarized light at right angles to the plane of vibration, suggests that it is scanning to maintain light reflection into both ocelli. If the mite turns as much as 45 degrees to either side one pigment cup can receive no light.

A model of the water mite's ocellus can be made from a water-filled florence flask and a tin can. A small area on the side of the flask is coated with Bon Ami to give a frosted surface, and is analogous to the photosensitive area of the eye. The tin can with both top and bottom removed is taped over the frosted side and serves as the pigment cup. If a microscope lamp is placed several feet to one side, at about a right angle to the tin can, and is aimed at the outer part of the "eye," a reflected image of the filament can be brought to sharp focus on the frosted area. With this arrangement, when a polaroid is put into the light beam and rotated, the image can be made alternately bright and dark. Eosin added to the water shows the light path clearly.

The cladoceran eye differs in several respects from the mite's eye. The single cladoceran eye is a cluster of ocelli, each protruding as a refractile hemisphere from a central mass of pigment. The lower lenses protrude especially far and are naked on their lower surfaces. The buried ends of the lenses do not complete spheres, but extend into the pigment mass as cones. While these conical bases probably represent an adjustment to the focal length of the lenses, they can also serve to increase sensitivity to polarization. Light may be reflected two or more times before reaching the sensitive area. Since the eye is not unlike a raspberry, with the blank area on the ventral side, the radially arranged ocelli enable the cladoceran to determine the plane of vibration of polarized light within narrow limits.

The observed orientation in Cladocera shows that, if the choice is given, the sides of the eye are kept darker than the front, top, or rear. If the animal swims at right angles to the plane of vibration in a vertical beam of light, an abundance of extraordinary rays are available for reflection into the front and rear lenses, but none for reflection into the lateral lenses. (Top lenses received direct illumination.) If the animal swims upright in a horizontally polarized lateral beam of light, an abundance of light is available for reflection into the top ocelli, but, again, none for the sides. (Front or rear lenses receive direct illumination.) The efforts of these organisms

to turn on their sides when a lateral beam of light is polarized vertically demonstrate the dominance of this light orientation over that to gravity.

If this orientation to polarized light is a matter of differential intensities, it should be easy to "fake" polarized light. This can be done by projecting a vertical beam into a very large beaker, which has pieces of white paper on opposite sides. The papers increase lateral reflection from two directions, and the population will immediately orient to them.

Light scattering material in the water under vertical polarized light produces a similar effect with scattered light most intense at right angles to the plane of vibration. The issue arises whether Cladocera respond directly to polarized light, or orient to intensity differentials due to light scatter in the water.

In one experiment the water was made so clear that no visible variation in lateral scatter was evident when a polaroid was rotated under a vertical light beam. After all of the reflection surfaces were blacked out, the cladoceran *(Daphnia magna)* oriented as well as ever. There is little doubt, however, that when both the extrinsic and the intrinsic sources of intensity differentials are present, they combine as stimuli for orientation.

The same mechanism for detecting polarization is feasible in the arthropod compound eye.[1] A single ommatidial cone represents a distinct advance over the cone-shaped internal reflection apparatus of the cladoceran eye. The cone of the ommatidium is composed of concentric lamellae, introducing multiple reflections and refractions, which should enhance the ability to detect polarization. In side illumination of an ommatidium in the appositional eye it is assumed that the refracted ray is absorbed by pigment cells, so that only the reflected light is perceived. While the superpositional eye would not detect polarized light so efficiently, the arrangement of the cone lens would still give it considerable ability to detect polarized light.

In view of the reported diurnal migration of pigment one would expect the sensitivity to polarized light to change from day to night. In either case, uniformly vibrating light should illuminate all ommatidia equally, whereas partially polarized light will result in uneven illumination, the rows of ommatidia at right angles to the plane of vibration gathering more light than those in the plane of vibration.

The value of the ability to perceive the orientation of polarized light is obvious in the bees, and not at all obvious in the mosquito larvae which show the same kind of response.

In the Cladocera two values of this ability are possible. One can be related to shafts of light passing surface vegetation and penetrating the water beneath. The scatter from such a shaft, which makes it visible from the side, is polarized with a horizontal plane of vibration. The orientation of the Cladocera to this polarization intensifies the light differential in the

[1]Since the preparation of this manuscript we have learned that similar conclusions have been reached by C. C. Stevens, M. Fingerman, and F. A. Brown, Jr., through studies on Drosophila.

eye between the front and sides, thus serving as a device for "homing" on the shaft. The value of getting into the lighted regions is probably related to food.

The second value relates to open water where discrete shafts of light are absent and skylight is unimpeded. Polarized skylight is most intense at right angles to the sun, and its prominence in the morning and evening has been found to cause orientation of experimental populations of Cladocera exposed only to the sky. Hence polarized skylight may serve as one cue in diurnal migration of open water plankton, even that of the deep "scattering layer" of the ocean. Such reactions would be especially interesting in relation to the specific responses to the wavelength of light that are being reported on these same organisms.

SUMMARY

Cladocera orient to polarized light by swimming at right angles to the plane of vibration. Similar behavior is described for other organisms. A mechanism for detecting the plane of vibration of polarized light is developed for the simple ocellus of the water mite, applied in detail to the cladoceran eye, and extended to the arthropod compound eye. The orientation by Cladocera is discussed in relation to discrete shafts of light in partly shaded ponds and to skylight in the open water.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

EXPERIMENTAL EVIDENCE OF SPECIES DISCRIMINATION IN THE SYMPATRIC SPECIES, *PEROMYSCUS TRUEI* AND *P. NASUTUS*

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Although reproductive isolation is fundamental to the modern biological concept of the species, there is little direct information concerning this phenomenon in most groups of animals. Morphological discontinuity, on which present-day classifications of species are mostly based, is obviously related to reproductive isolation. It seems likely, therefore, that in the great majority of cases the conclusion as to relationships of populations would be the same from either the morphological or the experimental approach. In other cases, relationships will be established satisfactorily only by application of experimental methods. For this reason it is important that methods be devised for testing the ability of animals of various groups to discriminate between their own and related populations, and it is important that these tests be applied in as many cases as is feasible.

The work here reported was done in June and July, 1942, in the Laboratory of Vertebrate Biology at the University of Michigan. At the time it was intended to be preliminary to an extensive study of species discrimination in the *Peromyscus truei* group. As the larger study has been indefinitely delayed, and as it is desirable to put on record all information bearing on reproductive isolation, the results of this study are presented here.

The methods used were those reported by Blair and Howard (1944). A male and a female of each of the two species being tested were introduced into a four-compartment cage. Daily records were kept of the distribution of the 4 mice in the four compartments until parturition by one of the females. These records are amenable to statistical analysis, and Blair and Howard (1944) have interpreted various of the combinations of individuals in terms of social behavior and species discrimination.

The mice tested were laboratory-raised descendants of animals collected by L. R. Dice in 1937. The stock of *Peromyscus truei* was collected at about 7500 feet elevation, about 8 miles northeast of Capitan, Lincoln County, New Mexico. Both *truei* and *nasutus* were taken there in the same ecological association (see Dice, 1942). The stock of *nasutus* was taken at an elevation of about 6100 feet, about four miles northwest of Lincoln, Lincoln County, New Mexico. Dice (1942) did not record *truei* from this station. The two species are sympatric throughout almost the entire range of *nasutus* and occur together at times in the same ecological associations.

Three experiments were run in which a male and female *truei* were tested against a male and female *nasutus*. Each experiment was ended by the

birth of a litter to the *nasutus* female. The female *truei* in Experiment 1 was obviously gravid when it was discontinued. No attempt was made to determine the male parent of the young born in the experimental cages. Fifteen combinations of the mice are possible in the experimental cage. The combinations that involve pairing of a male and female to the exclusion of the other mice should give the best evidence of whether or not there is species discrimination. Pairing of members of the same stocks may be interpreted as evidence that the mice do discriminate between the two stocks being tested. Cross pairing is evidence against discrimination. The frequencies of pair and cross-pair combinations in the three experiments and their totals are given in table 1. All of the other combinations

TABLE 1

FREQUENCIES OF SOCIAL COMBINATIONS OF A MALE AND FEMALE *Peromyscus truei* AND A MALE AND FEMALE *P. nasutus* IN A 4-COMPARTMENT POPULATION CAGE. OBSERVATIONS MADE ONCE DAILY. EACH EXPERIMENT DISCONTINUED UPON PARTURITION BY THE *nasutus* FEMALE.

Experiments	Evidence for species discrimination			Other combinations	Evidence against species discrimination			Totals
	Both paired	nasutus paired	truei paired		♀nasutus with ♂ truei	♀truei with ♂ nasutus	Both cross paired	
1	1	2	8	8	1	2	1	23
2	8	4	12	5	....	1	....	30
3	17	7	7	6	....	....	....	37
Totals	26	13	27	19	1	3	1	90
Expected	4.2	8.4	8.4	48.2	8.4	8.4	4.2	90.2

are pooled. Of these latter, the only ones that might provide evidence for or against discrimination are the combinations of one or the other male with the two females to the exclusion of the second male. This kind of behavior might indicate lack of discrimination and might result from one male dominating the other and preventing it from associating with the females (Blair and Howard, 1944). That such behavior was not of significant occurrence in the three experiments is indicated by the fact that such combinations occurred a total of only five times, although they would be expected to occur 8.4 times on the basis of chance.

The distribution of the mice in the compartments of the population cage were clearly not at random (table 1). The data from the three experiments are necessarily pooled for analysis because of the low expected frequency of each combination in the individual experiments. This pooling appears to be justified by the consistency from one experiment to the next. The pooled combinations that did not involve pairing or cross-pairing occurred significantly less frequently than would be expected under random distribution of the mice. Tested by a general chi-square formula, the chi-square value of 17.69 indicates that the negative deviation of these com-

binations from the expected was very significantly greater than would be expected by chance. Each of the three combinations involving cross-pairing of species occurred less frequently than would be expected under random distribution. If the three classes of cross-pair scores are pooled, the negative deviation from the expected is significant beyond the one per cent level, for the chi-square is 12.19. Tested similarly, the pooled pair scores occurred more frequently than would be expected under random distribution, for the chi-square is 96.43, which is very highly significant.

The low frequency of cross-pairing and the high frequency of pairing by species are taken to indicate that discrimination between members of the same and the other sympatric population is one of the mechanisms whereby the two populations are able to exist in the same region without losing their morphological discontinuity through interbreeding. Dice and Liebe (1937) found that male hybrids between the two species were sterile, but females were fertile when back-crossed to either species. The two species occur together in numerous ecological associations, although *truei* seems to be absent or scarce at the lower elevations at which *nasutus* occurs (Dice, 1942). Neither hybrid sterility nor ecological isolation would seem, therefore, to be a wholly effective isolation mechanism in the present case. They would presumably reinforce the action of species discrimination, which seems to be the most effective mechanism maintaining the discreteness of these 2 sympatric species.

SUMMARY

Mice of the sympatric species *Peromyscus truei* and *P. nasutus* from New Mexico were tested in a four-compartment population cage for evidence of species discrimination. Social combinations involving the pairing of a male and female of the same species occurred much more frequently than would be expected under random distribution of the mice in the cages. Combinations involving cross pairing of species occurred less frequently than would be expected. These results are interpreted as indicating that the mice of these sympatric species discriminate between members of their own and the other species, and this appears to be the principal mechanism that maintains the distinctness of the two species in nature.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

THE SURFACE RULE IN CRUSTACEANS[1]

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The question of the dependence of metabolic rate on body size, as expressed in Rubner's surface rule and related formulations, has found only in recent years systematic investigation outside the range of mammals. The present interest in this problem is partly due to the relation between "metabolic types" (that is, different forms of the size-metabolism relation) and "growth types" (that is, different forms of growth-in-time), as propounded by Bertalanffy (1941, 1951a; Bertalanffy and Müller, 1943). Three "metabolic types" (and corresponding growth types) have been distinguished, namely, proportionality of metabolic rate (1) to surface (ex.: fish and certain invertebrate classes); (2) to weight (ex.: insects); (3) intermediate between surface and weight proportionality (ex.: *Planorbidae*).

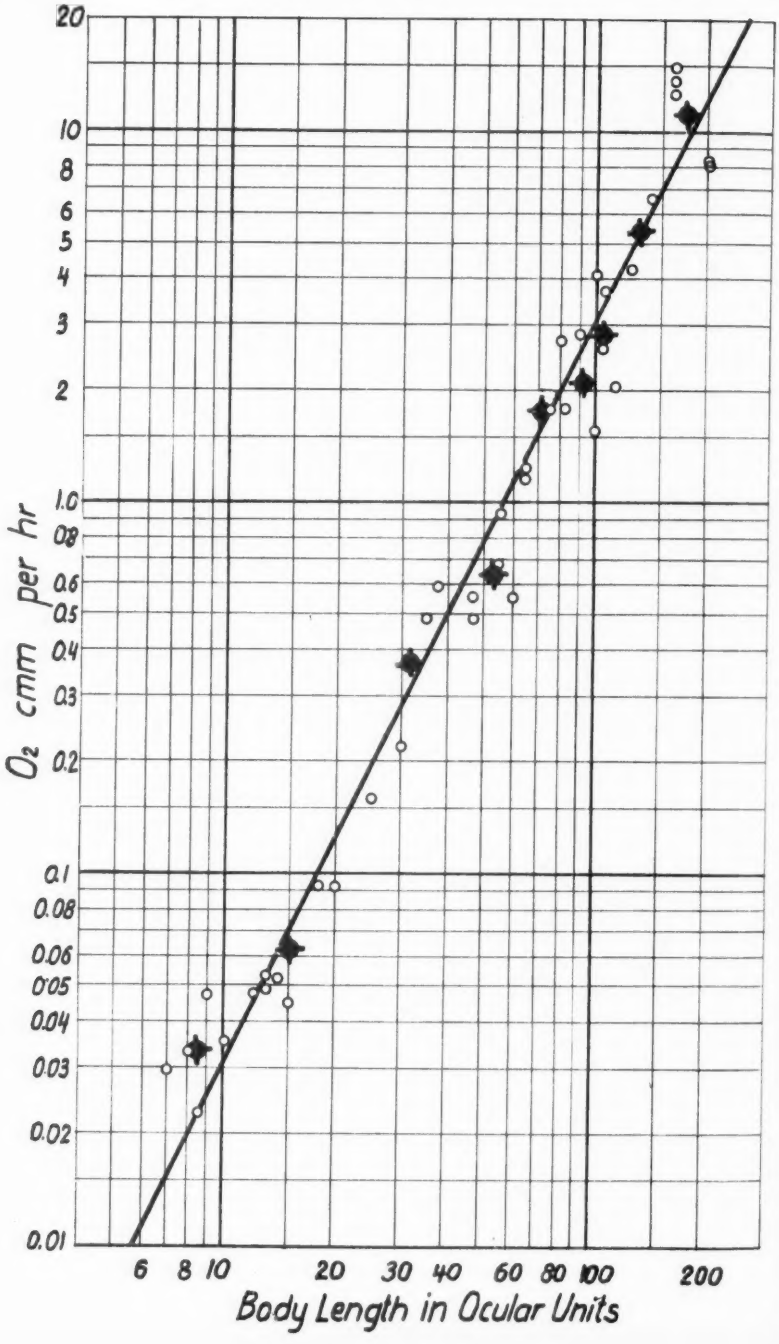
As far as crustaceans are concerned, the following data are available. In the order Branchiopoda, surface proportionality of metabolism was found in Daphnia (Jančařík, 1948). Also in Isopoda, surface proportionality was found in Asellus and Armadillidium (Müller, 1943). This was confirmed for Asellus and Oniscus, while Porcellio was stated to belong to the intermediate type (Will, 1952). In Decapoda, older data on *Potamobius astacus* (Kalmus, 1930) and *Potamobius torrentium* (Wolsky, 1934), suggesting surface or weight proportionality, respectively, are not very complete and reliable. In an extensive study *Pugettia producta* has been shown to belong to the intermediate type, that is, respiration is found to be proportional to the ¾ power of weight (Weymouth et al., 1944).

In the present work, *Artemia salina* (Branchiopoda) was studied. This species recommends itself for such investigations because of the ease of rearing and keeping it in the laboratory.

MATERIAL AND METHODS

The animals were obtained from commercial, air-dried egg cysts, hatched and cultivated in artificial sea water (composition according to Turtox Service Leaflet No. 20). The specific gravity of the filtered solution varies between 1.020 and 1.026. Individual batches were reared in finger bowls, their life history being carefully recorded. The animals were fed with fresh yeast and kept at room temperature (25–27° C). Oxygen consumption of all stages from the nauplius to the adult was measured by means of Kirk's microrespirometer ("Misco" model from Microchemical Specialities Co.),

[1]Supported by grants from the National Research Council and the National Cancer Institute of Canada.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0.03
0.02
0.01
O2 cmm per hr
6 8 10 20 30 40 60 80 100 200
Body Length in Ocular Units

submersed in a water bath of 30° C. The index droplet in the capillary of the microrespirometer consisted of kerosene stained with Congo Red. The calculation of the oxygen consumption was carried through by means of the formula indicated by Cunningham and Kirk (Glick, 1949). The number of animals used in one experiment varied according to their size, from 900 for the smallest, up to 1 for the largest specimens. Readings were taken every 10 minutes over a period of two hours. The length of the animals was measured by means of a micrometer eyepiece, and varied from 7 to 200 units, corresponding to 0.35–10 mm.

RESULTS

The surface rule of metabolism can be expressed by:

$$M = bS, \tag{1}$$

where M is metabolism, S surface, and b a constant. Surface can be considered as a square of length (L),

$$M = bL^2, \text{ or, taking logarithms, } \log M = \log b + 2 \log L. \tag{2}$$

Hence, the log-log plot of respiration against body length should give a straight line of slope $\alpha = 2$, if the surface rule applies. This presupposes that changes in body proportions (which, of course, do occur in larval development and progressive segmentation from the nauplius to the adult) can be neglected in comparison to the general trend of the quantitative dependence of respiration on body size.

In the case that the ¾ power rule of metabolism (Weymouth et al., 1944) applies,

$$M = b'W^{3/4}, \tag{3}$$

or, inserting $W = \text{Const. } L^3$,

$$M = bL^{9/4}, \log M = \log b + 2.25 \log L. \tag{4}$$

In this case, the slope would be $\alpha = 2.25$.

The present data (fig. 1) show that respiration is proportional to the square of body length, according to the surface rule. The relatively higher O_2-consumption of the nauplii is probably due to their lively movements and the mutual irritation of the large number of individuals to be used in these experiments. The range of variation, as studied in *Artemia,* is extraordinarily wide since variation from ⅓ mm. to 10 mm. length amounts to variation over more than five orders of magnitude in weight.

CONCLUSION

Oxygen consumption in *Artemia salina,* from the nauplius to the adult stage, follows the surface rule. Taking into account earlier results, it ap-

FIGURE 1. Respiration of *Artemia salina* in relation to body size. The sizes up to 9–10 units represent nauplii, those above 60 units adults. o : individual determinations, -✦-: means of body length and 0_2 for size classes (7-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-200 units). The theoretical line for surface dependence ($\alpha = 2$) is fitted to the means.

pears that surface proportionality of metabolism is found rather generally in crustaceans, although the relation between metabolism and body size especially in Decapoda requires further investigation of a larger number of species.

The demonstration of validity of the surface rule in certain invertebrate classes (for which the present work is a further example) is of importance for general physiology. It shows that the current explanations of the surface rule of metabolism (cf. Kleiber, 1947) which are based solely upon the investigation of mammals, cannot be considered as valid. This is true, in particular, for the classic explanation that the surface rule is a consequence of the energy expense needed for maintaining a constant body temperature (cf. Bertalanffy, 1951b).

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

NOTES ON A BREEDING SITE OF *Drosophila americana* NEAR ST. LOUIS, MISSOURI

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The close relationships of the species comprising the *virilis* group of Drosophila provide a situation which has been long recognized as especially suitable for the study of chromosomal evolution (Spencer, 1940; Patterson, Stone and Griffen, 1940; Patterson, 1941; Hsu, 1952). Thus far statistical analysis of populations of these flies has not been possible due to the difficulty of making consistently large collections. Specifically, in the case of the *americana* complex, individual collections of more than fifty flies have been only rarely reported (Patterson, 1942; Patterson and Wagner, 1943). An important contribution to the little-known ecology of wild members of the *virilis* group was the discovery by Spieth (1951) of pupae belonging to flies of the *montana* complex in the rotting phloem of aspen logs *(Populus tremuloides)* in Minnesota. An effort to gain a comparable understanding of the ecological relationships of flies of the *americana* complex has brought new information on the breeding habits of *D. americana.*

Repeated attempts to collect flies of the *Drosophila americana* complex in the St. Louis area during the summer of 1951 resulted in small numbers being taken in any given region despite the fact that, following previous leads, the traps were placed close to water. Over a three-month period the average number of these flies recovered per collection was less than two. The grand total numbered less than fifty. On October 5, 7 and 10, however, a series of nine cups containing fermented banana placed along the bank of the Meramec River in Jefferson County, Missouri, yielded 137 flies of the *americana* complex. Temperatures recorded at the time of these collections were 23, 12 and 14° C. respectively. The traps were suspended either immediately above the surface of the water or at a distance not greater than one foot from the water's edge in a grove of willows later identified by Dr. Edgar Anderson as sandbar willow *(Salix interior).* The willows lined the river bank for a distance of 300 to 400 yards in a narrow band not over 50 feet in width. As from mid-June until late-July, 1951, the area had been completely inundated by a prolonged flood, the bank was covered with five to six inches of soft mud and there was almost no vegetation other than the trees which were principally *Salix interior.*

Following the collecting activities on October 5th, an investigation of possible breeding sites was undertaken. Bark stripped from a stump of *Salix interior* approximately four inches in diameter revealed the presence of five Drosophila-like larvae. Shoots emerging from the stump indicated that at least the base of the stump was living. Placed on agar-cornmeal medium, the larvae pupated within 24 hours and all five were identified after emergence as belonging to the *americana* complex. The trunks and

branches of apparently healthy trees of the same species, ranging from three to eight inches in diameter, were also examined. Inspection revealed a relatively high frequency of slit-like separations of the bark three to ten inches in length, oriented in a longitudinal direction along the trunks and larger branches. The two lips of bark formed as a result of the split, although closely approximated to one another, demonstrated a consistent tendency to pull away from the underlying xylem. Although other species of willow were examined, these characteristic slits appeared to be present only on *Salix interior*. Underneath the bark near the space formed as a result of this condition, large numbers of pupal cases were discovered and a portion of these were collected for more detailed examination in the laboratory. A total of forty flies emerged from the puparia and all were identified as belonging to the *americana* complex.

A trip to the same collecting area was made on February 2, 1952, in an attempt to obtain information on overwintering. Stripping of bark adjacent to the slits resulted in the collection of a total of 67 *americana* complex pupae, of which 51 were recovered under a single bark separation. Except for a pronounced reduction in size the pupae were characteristic of the complex. Following retention in the laboratory at 25° C. for a period of 20 days, 16 cynipoid wasps emerged. These forms have been provisionally determined by Mr. L. H. Weld as Pseudoeucoila (Hexamerocera) sp. The reduced size of the puparia is apparently due to the infestation of the Drosophila larvae prior to pupation. Larvae collected from a stump of *Salix interior* at this time pupated in the laboratory and the adult flies which emerged were identified by Dr. H. D. Stalker as belonging to the genus Lonchaea.

Results of the investigation of *americana* complex populations in the St. Louis area seem to indicate a rather intimate relationship with *Salix interior*. While the true limitations of the ecological niche occupied by the members of this complex is yet to be determined the information now at hand provides at least a starting point. These findings, together with Spieth's contribution on the closely related *montana* complex, should not only lead to a more thorough understanding of the ecology of these flies but also open the way to a quantitative study of wild *virilis* populations.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

THE SURVIVAL AND DEVELOPMENTAL RATES OF *D. MELANOGASTER* AND *D. SIMULANS**

In most population cage competition experiments *Drosophila melanogaster* is superior to *Drosophila simulans*[1]. Since selective value is the resultant of a number of factors acting at different stages in the life cycle, experiments were carried out to compare the survival and developmental rates of these two species for the larval to adult period under similar and different nutritional conditions, in order to detect some of the factors responsible for their observed difference in selective value.

The Oregon-R wild-type stock of *D. melanogaster* and a wild-type stock of *D. simulans* from Japan were used. About 20 culture bottles were started at the same time for each of these stocks, each containing many parents. The parents were discarded after two days and the offspring were collected daily until few flies emerged. In these culture bottles, the top layer of the food, which had been churned by the larvae, was separated from the rest of the food pad and used in preparing different types of culture media. Three kinds of media were made. One type, M, contained equal amounts of used food from *D. melanogaster* bottles and of standard freshly prepared new food, another, S, had equal amounts of used food from *D. simulans* bottles and new food, while the third, N, was composed entirely of new food. The M and S media were prepared by bathing beakers containing the used food in hot water (60° C) for about five minutes so that the food became fluid. The new food, also fluid, was then added and the mixture stirred thoroughly. About 8 cc. of the M, S, or N media were poured into each one of a number of culture vials which were later seeded with live yeast.

In order to obtain larvae of the same age (± 1 hour), the large number of adults which had been collected were placed in a cellophane cylinder, the top of which was large enough to admit the neck of a culture bottle and the base of which fitted over the bottom of a large petri dish containing yeasted standard food medium. At the end of 2 hours several thousand eggs were laid, and either a new petri dish was placed into position for another collection of eggs or the adults were put into culture bottles for reuse the following day. Eggs were collected, in this manner and at the same time, for

*This work has been supported by grants directed by H. J. Muller from the Rockefeller Foundation and the United States Atomic Energy Commission (Contract AT (11-1)-195).

both species. Twenty-four to 28 hours after egg laying started, larvae hatched from these eggs were transferred at random to an M, S, and N vial until there were 100 in each culture. This procedure was followed until there were 9 cultures for each type of food for each species. Once eclosion started, the adults were removed from the vials and counted every day, whenever possible, until no additional offspring emerged. All work was done at 25 ± 1° C.

RESULTS

While 2149 of the original 2700 *D. melanogaster* larvae, or 80 per cent survived to eclosion, this is true only for 1406 of an equal number of *D. simulans* larvae, or 52 per cent. The percentage of the survivors which emerged before the first count and between successive ones is given in table 1. Of the 900 *D. melanogaster* larvae placed in each of the M, S, or N foods the number of survivors was 723, 704, and 722, respectively. Correspondingly, only 552, 346, and 508 of the initially identical number of *D. simulans* larvae survived these treatments.

TABLE 1

DEVELOPMENTAL TIME AND NUMBER OF SURVIVORS FOR THE YOUNG LARVA TO ADULT STAGE

Drosophila species	Initial no. larvae	No. obtained as adults	Per cent of survivors obtained in count no.						
			1	2	3	4	5	6	7-11
melanogaster	2700	2149	14.1	58.2	11.2	8.1	3.5	2.7	2.2
simulans	2700	1406	8.3	45.8	26.0	14.3	3.7	0.8	1.1

DISCUSSION

A major factor in the smaller percentage of *D. simulans* than *D. melanogaster* larvae to survive to the adult stage is the readily observed fact that mature larvae of the latter but not of the former species move out of the food of culture bottles and vials in order to pupate. *D. simulans* pupae are almost always found on the surface of the food, which becomes soft from the churning of the larvae, and a considerable number of pupae are either submerged in the food or displaced so that successful eclosion is made impossible or more difficult. The smaller number of *D. simulans* survivors from the S as compared with the M and N cultures is considered to be due to this factor acting in conjunction with an S medium which probably became fluid more easily than the other two. The similarity in the number of *D. melanogaster* survivors on all types of media, true also for the *D. simulans* larvae on M and N foods, leads to the conclusion that the biological by-products associated with *D. melanogaster* and *D. simulans* larvae, under the present experimental conditions, do not have a marked effect on survival.

D. simulans survivors are not only fewer than those of *D. melanogaster* but they require a longer period of time to develop from larva to adult (table

1). Whereas 14.1 per cent of *D. melanogaster* survivors have emerged by the first count, only 8.3 per cent of *D. simulans* have, and this superiority of *D. melanogaster* is evident also in the second count. Although breeding conditions might be different in natural populations, it is likely that these differences in survival and developmental rates are important components of the selective values of these species when competing in population cages.

SUMMARY

In laboratory cultures, the number of newly hatched larvae which survive to the adult stage is greater and the time required to complete this development is shorter for *D. melanogaster* than for *D. simulans*. These differences are probably important factors in the superiority of *D. melanogaster* to *D. simulans* in population cage competition experiments.

The data do not support the idea that food in which larvae have previously grown contains a factor biochemically influencing the survival of a succeeding generation of larvae.

I wish to thank Miss Julia Colvin and Mr. Seymour Abrahamson for their capable technical assistance.

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SEX DIFFERENCES OF CROSSING-OVER IN CLOSE LINKAGE

In discussing "Variations in crossing-over" Hutt[1] (p. 497) presents a table (his Table 63) of five recombination fractions observed in poultry with sufficient numbers in both sexes. He concludes: "Apparently crossing over is influenced very little by sex in the fowl, at least so far as these figures for three different linkage groups can be any guide."

The table is taken from a note by Warren[2] and requires annotation on two points. (a) The ratio for Rose comb and Creeper in females is given as 0.54 ± .70%, and should be 0.54 ± .07%, as in Warren. (b) Warren has given *probable* errors and not standard errors for both figures. The net result is that for this close linkage the sex difference, 0.54 in females and 0.24 in males, is quite significant statistically as indeed Warren[2] had recognised. Landauer[3] had previously pointed to the difference as suggestive. On the data as compiled by Warren the χ^2 value is 5.497, or 4.760 with Yates' adjustment, giving P less than .03, for a deviation so large in either direction.

Attention is drawn to this case owing to its parallelism with what has been found at Cambridge in recent linkage determinations in the house mouse, namely, that significant differences due to sex, as also to age, may be detected in *short* chromosome segments, and that in these cases rather high ratios in crossover frequencies are apparent.

When several short segments can be tested the surprising fact appears that there may be a sharp contrast in the reactions even of short adjacent segments. For example, in the fifth linkage group of the house mouse a simultaneous test with the four loci Agouti (A), undulated (un), wellhaarig (we), and pallid (pa) shows recombination as follows:

	A - un	un - we	we - pa
In females	4.67 ± 0.04	7.39 ± 0.07	4.20 ± 0.04 per cent
In males	4.60 ± 0.04	4.44 ± 0.04	2.24 ± 0.02 per cent

These are based on 2503 offspring of four-fold heterozygous females, and 2499 from corresponding males.

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PATTERN FORMATION IN CONCENTRATED PARTICULATE SUSPENSIONS

Concentrated cultures of motile protozoa and algae[1,2,3,4] and spermatozoa[5], under suitable conditions, have been observed to form rather characteristic patterns in liquid media.

We have used motile bacteria, such as *Escherichia coli* and *Pseudomonas fluorescens*, and have found that dense cultures of these form patterns very similar to those we observed with Tetrahymena. The pattern is of much finer texture, consists of smaller units, and is slower in forming. It can best be observed by centrifuging bacterial cultures and suspending this concentrate in a layer several millimeters thick in a Petri dish. This general type of pattern is formed as long as the motility of the organisms is not markedly reduced.

Further experiments designed to elucidate the relationship of motility to this phenomenon indicated, in contradistinction to the results obtained with the much larger protozoa and algae, that heat-killed bacteria also formed patterns comparable in many respects to those of live motile cultures. Red blood cells and other non-motile bacteria, such as *Micrococcus aureus*, did likewise.

We were also able to demonstrate pattern-formation with a hydrophilic colloid.† The design was similar to that obtained with non-motile or dead bacteria. If one places the suspension in an insulated container so that thermal gradients are minimized or prevented, patterns do not form in suspensions of non-motile or killed cells. Under similar conditions motile organisms may form diffuse patterns as long as movement continues.

It is evident from these extended observations that pattern formation is a more widespread phenomenon than previously realized. The types of patterns formed by the different systems noted vary in accordance with the factors primarily concerned in their formation. These may be entirely physical, as in the inert colloidal system, or mainly biological, as in the systems with powerfully swimming organisms, or the pattern may result from a more nearly equal combination of biological and physical forces, as in bacterial suspensions.

Important physical factors concerned appear to be convection currents, thermal diffusion resulting from surface evaporation, unequal heat distribution in the medium, and possibly others, which contribute to a quasiturbulence within the system. There is soon an unequal dispersion of particles and the situation is further complicated by the osmotic pressure gradients which result. Among factors exerting a minor, although detectable, effect on the induction of currents, may be listed: changes in surface charge and solvation resulting from rapid swirling, variation in size of particles, heat of mixing and diffusion following agitation. The pattern which results from

†The material used was *Aquadag*, a product of Acheson Colloids Corp., Port Huron, Michigan.

purely physical forces, although of complex origin, is less complicated than that formed by motile organisms, since there is no independent movement involved. In the latter all the physical factors apply, but their relative influence is dependent on the size of the particles involved. In large forms such as the protozoa, the physical forces exert a negligible influence, since a loss of motility precludes pattern formation.

As particles decrease in size toward colloidal proportions, the physical forces exert an increasingly important influence to which, in living systems, must be added the biological factors, such as motility itself, heat resulting from movement, and heat of metabolism. Motility contributes relatively less to pattern formation as particle size decreases.

Once established, patterns of the several types described above, remain intact some time. The type is determined by the relative interplay of the various causative forces. Patterns appear to be highly dynamic, with individual particles or organisms surging around in a centrifugal manner on the periphery of rather sharply outlined globular masses in which the lines of demarkation are continually changing. The concentration gradients established as a result of these currents become markedly accentuated when free-swimming organisms are involved. Taylor[6] has shown mathematically that when motile organisms are sufficiently close to one another, their flagella tend to beat in phase, thus reinforcing the waves produced, and that such forms tend to orient themselves in one direction. Thus, once a concentration gradient has been established as a result of the many factors involved, the organisms, if sufficiently large, tend to assume control of the current by a wave reinforcement process such that the individual cellular velocity due to motility is considerably exceeded by that of the rotating globular mass. Any treatment which affects motility adversely tends to cause this type of pattern to approach the more diffuse inert colloidal type.

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Vol. LXXXVII, No. 833 The American Naturalist March-April, 1953

BOOK REVIEWS

THE THEORY OF THE GENE

The first symposium on the problem of the gene was organized by M. Demerec at the Long Island Biological Laboratory in Cold Spring Harbor, N. Y. in 1941. The resulting volume on "Genes and Chromosomes"[1] gave an account of the status of the theory of the gene at that time. Very fittingly, another symposium on the same problem was held under the same auspices ten years later. The volume on "Genes and Mutations,"[2] issued in the summer of 1952, reflects the development of the gene theory during the decade intervening between the two symposia. During this decade the advances in genetics bearing on the theory of the gene have been very considerable. Nevertheless, the uncertainties are many. Quite apart from the lunatic fringe of Lysenko and his like, serious and even radical differences of opinion exist among competent geneticists concerning the very foundations of the gene theory. Such differences are manifestly present among the participants in the symposium. The volume on "Genes and Mutations" contains 35 articles by 54 authors. It is neither necessary nor desirable to review the contents of these articles one by one, especially since some of them are data papers which could have been published as well in current genetical and other periodicals. It is more useful to discern in the symposium the broader trends of the modern genetic research and thinking.

Some 15 years ago, R. Goldschmidt startled his colleagues by denying the existence of the gene, and soon thereafter by his saltational theory of evolution. Although Goldschmidt has somewhat moderated his stand during the intervening years, his opening article in the symposium still expresses, with the customary vigor and brilliance, his now familiar revolutionary views. Goldschmidt's central argument remains that the phenomena of position effects make the idea of discrete and independent genes untenable. A closer examination shows however that what Goldschmidt is rejecting is actually the one gene—one enzyme theory, which he states thus: "Because a certain step in the synthesis of a genetically controlled product is prevented to occur by the mutant, presumably via the absence of the specific enzyme for that step, it is concluded that a normal gene exists which controls the production of this enzyme." Now, the only article in the symposium volume which still espouses the one gene—one enzyme theory is that of Horowitz and Leupold, and even they state this "hypothesis" in a different way, namely: "The concept is that of a gene whose sole activity aside from self-duplication is that of functioning in the synthesis of a particular enzyme or enzyme precursor." It seems to the present writer that the reservation "aside from self-duplication" makes the whole hypothesis meaningless, because self-duplication is evidently the most essential function of every gene. A gene which reproduces itself

does so at the expense of some materials in the cell, and the by-products of the reproduction process may not be without influence in the metabolism of the cell in which this process takes place. Thus a gene may function, and function differently in different tissues, regardless of whether we are able to detect a particular enzyme for which one of the alleles of the gene is responsible. Bonner, as well as Lederberg et al., is no less sceptical than Goldschmidt regarding the one gene—one enzyme view, nevertheless without following him to his other conclusions.

Goldschmidt's position does, however, gain partial support from McClintock, whose article, the longest in this volume, gives the most adequate account to date of her important findings concerning the phenomena of chromosome dissociation and of mutable loci in maize. As a piece of experimental research the work of McClintock is altogether admirable. A cytogenetic situation which is perhaps more complex and recondite than any yet discovered in genetics is analyzed with a highest degree of craftsmanship and ingenuity. The bearing of this work on the theory of the gene is a different story. McClintock is doubtless right when she recognizes that the remarkable phenomena which she has discovered in maize belong to the same category as those of mosaicism known for about two decades in Drosophila, particularly of mosaicism associated with chromosome breakage and with shifts in the position of genes with respect to heterochromatic sections of the chromosomes. What McClintock is dealing with, then, are position effect phenomena, which are regarded as contradicting the theory of the gene by Goldschmidt but not by most other geneticists. This is, in effect, recognized by McClintock herself in the following statement, with which the present writer can only agree: "It will be noted that use of the term gene has been avoided in the foregoing discussion of instability. This does not imply a denial of the existence within chromosomes of units or elements having specific functions. The evidence for such units seems clear.... It cannot be denied, in the face of such evidence, that certain loci or regions in the chromosomes are associated in some manner with certain cellular reactions or with the development of particular phenotypic characters. This is not the major questionable aspect of current gene concepts. The principal questions relate to the mode of operation of the components at these loci, and the nature of the alterations that affect their constitution and their action." What is, then, the utility of avoiding the use of the term "gene"? This avoidance results merely in verbal contortions which are not conducive of clarity of either thought or exposition. As to the mode of operation of genes and gene components, not even the partisans of the one gene—one enzyme theory claim to have discovered all the answers. The genes are the "units" of chromosome organization the existence of which is recognized by McClintock, as well as by a great majority of other geneticists.

The papers of Stadler, Stephens, Bonner, and Lewis investigate the delimitation of certain genes in maize, cotton, Neurospora, and Drosophila respectively. These authors successfully hold the middle ground between

the scepticism of Goldschmidt and McClintock on one side and the Horowitz and Leupold's over-simplification on the other. Stadler, with his customary almost forensic finesse, discusses the methods of experimental study of the gene through examination of its mutational variability. He shows that most favorable materials for such study are offered by genes the phenotypic effects of which show a large "variety of attributes in which valid comparisons of allelic forms may be made." The *R* and *A* genes in maize fulfill this requirement extremely well, and the examination of their mutations shows that "what is taken to be a single gene on the basis of transmission experiments consists of a complex of independently mutating elements." The same is true for the *R* and *C1* genes in cotton studied by Stephens. The classical mutations in Neurospora have properties opposite to those considered by Stadler to be requisite for successful analysis of the gene structure, but Bonner shows that some of the genes in that organism do have qualities similar to the maize and cotton genes studied by Stadler and Stephens.

The decade 1941–1951 has witnessed rapid and vigorous growth of the genetics of microorganisms, of biochemical genetics, and of evolutionary genetics. The bearing on the theory of the gene of the advances in the first and the second of these fields has been recognized in the symposium more than that of the third. The section of the symposium entitled "Evolution of the Gene" contains only four papers, one of which, the otherwise very valuable work of Schultz and Redfield, has no relation to this topic, and little to the theme of the symposium as a whole. Yet it should be evident that the structure and the functioning of the genes are products of age-long evolutionary development controlled by natural selection, and are not comprehensible except in the evolutionary context. Stephens and Lewis consider this basic problem in connection with closely linked genes having similar developmental effects. Such partially allelic, or pseudo-allelic, genes apparently arise through duplication of previously existing genes, and provide materials for the origin of new genes through divergent mutational changes. The details of this process of divergence are yet to be clarified. Stephens supposes that in the case of the *C1* genes in cotton the divergence has occurred by a reshuffling of functions which were already present in the original gene. This may very well have happened in this particular instance, but such a view can hardly be generalized, because this would mean an evolutionary trend towards less and less differentiated genes, which does not seem particularly probable. Perhaps the suggestion that diverging duplicate genes may come to control successive steps in biochemical reaction chains will prove fruitful.

Only a single paper dealing with particulate components of the cytoplasm was presented at the 1941 symposium, and that paper considered these components more as chemical than as genetic entities. The 1951 symposium has cytoplasmic heredity and its relations to the nuclear genes as one of its main topics. Five important papers of the section entitled "Cytoplasmic Constituents of Heredity," as well as the closing paper of

the Symposium by Sonneborn, are devoted to this topic. It is now clear that the reluctance felt until recently by some of the more conservative geneticists to admit the existence of genetic units in the cytoplasm was unfounded. More than that, studies on plasmatic heredity are beginning to throw light on some of the most fundamental genetical and general biological problems, such as the problem of cell and tissue differentiation in the individual development, and that of the relationships between pathogens, symbionts, and the orthodox carriers of heredity. As stated by Sonneborn, "It is particularly important to recognize that both nuclear and cytoplasmic conditions are self-perpetuating, and, in a sense, mutable. If the effective gene dosage changes, the concentration of active cytoplasmic substance changes and the cycle is established on a new level.... The fact that in this system of interdetermination perpetuation of an altered condition in nucleus or cytoplasm is clearly not due to direct copying of a model may at first give the impression that it has nothing to do with self-duplication and mutation of genes. This impression is however merely a reflection of the strength of our preconceptions in thinking about the gene and mutations and is not due to any difference in standards of judgment or to any known difference in the two sets of phenomena."

It is a very similar preconception that has recently led to some fruitless polemics concerning the existence of "plasmagenes."[2] L'Heritier is clearly right that it is "hopeless to draw the line between plasmagenes and viruses on such criteria as pathogenicity or power of diffusion." It happens that the failure of all attempts to draw such a line is perhaps the most important result to date of the studies on cytoplasmic heredity. It points to the conclusion that plasmagenes and viruses are merely terminal links in a chain of evolutionary transformations. Furthermore, it seems very probable that such transformations are in at least some cases reversible. One may think that natural selection would in general tend to minimize the disoperation and to encourage cooperation among representatives of the same and of different species. Changes in the genetic constitution may occur in both host and pathogen populations. These changes will generally lead to mutual tolerance, commensalism, symbiosis, and finally to mutual dependence. This evolutionary trend may occasionally be reversed. On the phylogenetic level such reversals would give rise to new infectious diseases, on the ontogenetic level they may result in neoplastic growths.

In her important paper on chemical mutagenesis, Auerbach quotes the statement made by Muller at the 1941 symposium, to the effect that "attempts to induce mutation by chemical means had not yet given any clear positive results." At the 1951 symposium the articles dealing with chemically induced mutations, and with interrelations between radiation and chemical mutagens, formed one of the largest sections. It is natural that most work on chemical mutagenesis has so far been exploratory and empirical. Generalizations and broader principles are yet to emerge. Nevertheless, some very promising leads have already been found. Here belong the discovery of delayed effects of some chemical mutagens in Drosophila

(Auerbach), inequalities in the effects of the same mutagen in different organisms and in different tissues of the same organism (Auerbach, Westergaard and his collaborators), of the proportionality of the mutation rates in certain bacteria to time rather than to the number of generations (Novick and Szilard), and the progress on the road towards the induction of specific mutations in specific genes (Ephrussi-Taylor).

The successes attained during the last decade in the fields of genetics of bacteria and of bacteriophages are spectacular. The work in these fields may be considered from at least two points of view. First, it has been demonstrated that the genetic mechanisms in the primitive forms of life are more nearly similar to those discovered by classical genetics in higher organisms than most geneticists would have dared to suppose a decade ago. Secondly, microorganisms are obviously more suitable as materials for investigation of certain genetic problems than the conventional animal and plant species used by earlier geneticists. Here belong, first of all, many problems of mutagenesis and mutation rates. From the first point of view, the work of Lederberg and his colleagues is outstanding. Not only have gene segregation and recombination been discovered in certain bacteria, but linkage groups, genetic chromosome maps, nondisjunction, and translocations are established or suspected. Even if, as seems possible, some of these phenomena in bacteria will prove not to be comparable to their Drosophila prototypes, we can be sure that the basic mechanisms of heredity are very nearly universal in the living world.

The developments of the theory of the gene in recent years have unquestionably been rapid and impressive. No less certainly, the theory of the gene has not yet congealed into a body of schoolbook dogma. This will perhaps be discouraging to those who like completeness and stability in science. Others will find inspiration and encouragement in the fact that the edifice of the gene theory is far from finished, and in the realization that builders are needed.

LITERATURE CITED

[1]Genes and Chromosomes, Structure and Organization. 1941. Cold Spring Harbor Symposium on Quantitative Biology, 9: I–X, 1–315.
[2]Genes and Mutations. 1951. Cold Spring Harbor Symposium on Quantitative Biology, 16: I–XVI, 1–521.

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NEW YORK CITY
November 1, 1952

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

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New York 71, N. Y.

Agar, W. E., 1951 (2nd edition revised). A contribution to the theory of the living organism. 235 p., $3.75. Melbourne University Press (27/6); Cambridge University Press, New York.

Annals, Marie Curie-Sklodowska University, Lublin. Section C, Biology. Vol. 6, Nos. 5-14, 1952.

Blacker, C. P., 1952. Eugenics: Galton and after. 349 p., ill., $5.00. Harvard University Press.

This book by the long time general secretary of the Eugenics Society (London) was published in England in 1950. It gives a general and readable account of the life and views of Sir Francis Galton (1822-1911), the founder of Eugenics, and of some of the developments in genetics, demography, and other fields of Galton's interests which have taken place since his death. Two appendices contain Galton's views on race and a bibliography of Galton's publications.

L.C.D.

Lillie, Frank R., 1952. Lillie's development of the chick, an introduction to embryology. 3rd edition, revised by Howard L. Hamilton. 624 p., ill. Henry Holt and Company, New York.

This revised edition brings up to date one of the most important classics for the study of embryology at all stages of development. The original ground structure of the book has been preserved as well as much of the text and nearly all of its excellent illustrative material. Additions include among others a table of normal development with description as well as excellent illustrations of individual stages, and a chapter on the development of the integumentary system. The most important changes of this edition are based on the integration of results of recent experimental work with those of earlier descriptive studies for an account of chick development. In early stages such changes concern particularly the account of entoderm formation, dynamic events centering around the primitive streak, prospec-

tive potencies of the blastoderm, and, in later stages of organogenesis, the nervous system, urogenital system, integument, skeleton. The list referring to the literature for each chapter has been extended and brought up to date and encourages as well as facilitates consultation of original papers on different subjects. Altogether this "introduction to embryology" meets the highest standards in every respect and represents an essential part of the literary equipment of not only every embryologist but of every biologist as well.

S.G.W.

McKeown, Keith C., 1952 (2nd edition, revised and enlarged). Australian spiders, their lives and habits. 274 p., ill., 21 shillings. Angus and Robertson, Ltd., Sydney and London.

Mr. McKeown has been collecting and observing Australian spiders for many years, and he has succeeded in getting the collaboration of amateur observers in many parts of the continent. The result, here brought together, is a comprehensive natural history of the spider fauna containing much material that should interest a wide variety of naturalists all over the world. The style is sometimes a little strained toward the "popular" and the long quotations from letters are sometimes tedious; but the book is a mine of out-of-the-way information. There are many striking photographs.

M.B.

Preston, R. D., 1952. The molecular architecture of plant cell walls. 211 p., ill., $6.75. John Wiley and Sons, Inc., New York.

A highly interesting book written to satisfy the demand for a modern account in English of the fine structure of plant cell walls. Chiefly intended for botanists, it is of value also to fiber technologists and physicists, and based on recent literature and original investigations. Cell walls are discussed from the point of view of chemical nature, physical investigation methods, and individual wall type structure. The properties of the primary walls of growing cells are examined, and the concluding, though brief, section deals with the mechanism of cellulose orientation and growth. This raises questions significant for the student of development and morphogenesis; it discusses in particular the inadequacy of known physical principles and simple mechanical explanations to account for the orientation in growing walls and for the relation between wall structure and cell shape. The mechanism involved in orientation of cellulose and its manifestations in growth must be looked for at some fundamental molecular level in protoplasm, where it may be related to an organized system in the cytoplasmic proteins. Thus the account, which began as a study of the static structure of mature and dead cell walls, at the end points logically toward a more dynamic approach and study of protoplasmic activity in growing cells.

R.B.

Protein metabolism, hormones and growth, a symposium, 1953. Six lectures presented at the eighth annual conference on protein metabolism, Bureau of Biological Research, Rutgers University, February 1-2, 1952, by Leo T. Samuels, James H. Leathem, Charles D. Kochakian, Frank L. Engel, Jane A. Russell and Laurance W. Kinsell.

Ramsay, J. A., 1952. Physiological approach to the lower animals. 148 p., ill., $2.75. Cambridge University Press, New York.

This book is "intended for the student at the beginning of his university career." While it may not be adapted to use in any particular course now given in American universities, it deserves careful study by all teaching biologists both because of content and style. The idea of using invertebrates for an introduction to certain principles of physiology has many possibilities, especially since Ramsay shows clearly that the physiological processes can be explained and understood without recourse to the elaborate gobbledegook of invertebrate morphology. The style of the book—clear and simple without being simple-minded—merits careful study by all text writers.

M.B.

Shirley, Hardy L., 1952. Forestry and its career opportunities. 492 p., ill. $6.50. McGraw-Hill Book Company, Inc., New York.

The emphasis is upon applied forestry and on the activities and duties of foresters. Included, however, are brief accounts of the history and of the broader scientific aspects of forestry.

C.Z.

Sjögren, Torsten, and Tage Larsson, 1949. Microphthalmos and anophthalmos with or without coincident oligophrenia. 103 p., 8 figs., 20 tables. Acta Psychiatrica et Neurologica, Supplementum 56, Ejnar Munksgaard, Copenhagen.

Sjögren, Torsten, Hakon Sjörgren, and Åke G. H. Lindgren, 1952. Morbus Alzheimer and Morbus Pick. 152 p., ill. Acta Psychiatrica et Neurologica Scandinavica, Supplementum 82. Ejnar Munksgaard, Copenhagen.

Tarp, Fred H., 1952. A revision of the family Embiotocidae (the surfperches). 99 p., ill. Fish bulletin No. 88. State of California, Department of Fish and Game, Bureau of Marine Fisheries.

University of South Carolina Publications, Biology, Series III, Vol. 1, No. 1, November, 1952. Research Committee, University of South Carolina, Columbia, S. C.

Penny, J. T., Distribution and bibliography of the amphibians and reptiles of South Carolina, pp. 3-28.

Freeman, H. W., Fishes of Richland County, South Carolina, pp. 28-41.

Waddington, C. H., 1952. The epigenetics of birds. 272 p., ill. $7.00. Cambridge University Press, New York.

Epigenetics is defined by the author, who is professor of animal genetics at the University of Edinburgh, as "the science concerned with the causal analysis of development." In this book it is concerned chiefly with some aspects of early morphogenesis in the chick, with a chapter on some genetic effects and their phenocopies in the fowl. This book is rather a review of literature than an attempt at synthesis of a field. The new hybrid name applied to experimental embryology is likely to bring more confusion than clarification to the study of development.

L.C.D.

Waksman, Selman A., 1952. Soil microbiology. 356 p., ill. $6.00. John Wiley and Sons, Inc., New York, N. Y.

An excellent treatment of soil science by a 1952 Nobel laureate, who has long been world famous for his basic discoveries in antibiotics. The book really covers more than its title indicates in spite of the fact that its title stakes out a very broad claim.

C.Z.

Wardlaw, C. W., 1952. Morphogenesis in plants. 176 p., ill. $2.25. John Wiley and Sons, Inc.

A contribution to the recent renaissance of plant morphogenesis. "The aim has been to give some idea of the contemporary outlook on morphogenesis in plants, to indicate its multi-aspect character, to illustrate by selected examples what has been achieved and the scope for further investigations." The aim has been accomplished.

C.Z.

Womersley, H., 1952. The scrub-typhus and scrub-itch mites *(Trombiculidae acarina)* of the Asiatic-Pacific region. Part 1 (text) 435 p. £2 2s. Part 2 (plates) 118 plates £1 1s. Records of the South Australian Museum, Adelaide, S. A.